UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

Date: March 4, 2013

SUBJECT: Cyantranilliprole: Data Evaluation Reports (DER) for toxicology studies on cyantraniliprole. Global Joint Review (GJR) –EPA: primary reviewer.

PC Code:090098
Decision No.: 451670
Petition No.: 1F7894NA
Risk Assessment Type: Aggregate human health
TXR No.: 0056591
MRID No.: See the listing in the memo.

DP Barcode: D404762
Registration No.: 352-IAE, 352-IAG, 352-IAI, 352-IAL, 253-IAN, 352-ILA, 352-ILI, 352-ILO, 352-ILT, 100-RUEE, 100-RUEG, 100-RUEN, 100-RUER, 100-RUEU, 100-RURI
Regulatory Action: Section 3 Registration
Case No.: NA
CAS No.: 736994-63-1
40 CFR: to be determined (new active ingredient)

FROM: Whang Phang, PhD
Risk Assessment Branch III
Health Effects Division (7509P)

THROUGH: Christine Olinger, Branch Chief
Risk Assessment Branch III (RAB3)
Health Effects Division (HED; 7509P)

TO: Thomas Harris, Risk Manager Reviewer
Registration Division (7505P)

Cyantraniliprole is a global joint review (GJR) pesticide; EPA is the primary reviewer for toxicology studies which consisted of 52 main studies and 6 supplemental studies. The MRID number of each supplemental study is underlined. The DER for each main toxicology study has been prepared by US EPA and evaluated by the global partners (Australia, Canada England, and France). The data from each supplemental study are incorporated into the appropriate main study. All the DERs are in the OECD format except the 28-day inhalation study. The conclusion presented in each DER has been confirmed by all the partners. The study citation and its corresponding MRID number are presented below. The paper copy of each DER is attached.

MRID	Citation Reference
46979931	Myhre, A. (2006) IN-F6L99: Bacterial Reverse Mutation Test. Project Number: 20597, 16784, 500. Unpublished study prepared by E. I. Du Pont de Nemours and Co., Inc. 52 p.

MRID	Citation Reference
48119938	Nabb, D. (2010) Cyantraniliprole (DPX-HGW86) Technical: Repeated-Dose Oral Toxicity 2-Week Gavage Study in Rats with Metabolism and Genetic Toxicology. Project Number: DUPONT/13430/OCR, 13430, 1583. Unpublished study prepared by E.I. du Pont de Nemours and Company, and Experimental Pathology Laboratories. 130 p.
48119940	Carpenter, C. (2009) DPX-HGW86 Technical: Repeated Dose Oral Toxicity 28-Day Feeding Study in Mice. Project Number: DUPONT/15205/OCR, 15230, 881. Unpublished study prepared by E.I. du Pont de Nemours and Company. 250 p.
48119941	Carpenter, C. (2009) DPX-HGW86 Technical: Repeated Dose Oral Toxicity 28-Day Feeding Study in Rats. Project Number: DUPONT/15206/OCR, 15230, 880. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 248 p.
48119942	Luckett, E. (2007) DPX-HGW86: 28-Day Oral Palatability Study in Dogs. Project Number: DUPONT/15456/OCR, 125/052, 15877. Unpublished study prepared by MPI Research, Inc. 356 p.
48119943	MacKenzie, S. (2007) DPX-HGW86 Technical: Subchronic Toxicity 90-Day Feeding Study in Mice. Project Number: DUPONT/16992/OCR, 15850, 1318. Unpublished study prepared by Experimental Pathology Laboratories, Inc., and E.I. du Pont de Nemours and Company, Inc. 418 p.
48119944	MacKenzie, S.; Gannon, S. (2011) DPX-HGW86 Technical: Subchronic Toxicity 90-Day Feeding Study in Mice. Project Number: DUPONT/16992/OCR, 15850, 1318. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 26 p. *(Supplemental study of MRID 48119943)*
48119945	Carpenter, C. (2007) DPX-HGW86 Technical: Subchronic Toxicity 90-Day Feeding Study in Rats. Project Number: DUPONT/16993/OCR, 15850, 1026. Unpublished study prepared by E.I. du Pont de Nemours and Company. 610 p.
48119946	Carpenter, C.; Gannon, S. (2011) DPX-HGW86 Technical: Subchronic Toxicity 90-Day Feeding Study in Rats. Project Number: DUPONT/16993, 15850, 1026. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 34 p. *(Supplemental study of MRID 48119945)*
48119947	Luckett, E.; Gannon, S. (2009) DPX-HGW86 Technical: 90-Day Dietary Toxicity Study in Dogs. Project Number: DUPONT/16994/OCR, 125/055, 15850. Unpublished study prepared by E.I. du Pont de Nemours and Company, and MPI Research, Inc. 24 p. *(Supplemental study of MRID 48119948)*
48119948	Luckett, E. (2007) DPX-HGW86 Technical: 90-Day Dietary Toxicity Study in Dogs: Final Report. Project Number: 15850/OCR, 125/055, 1319. Unpublished study prepared by MPI Research, Inc. 724 p.
48119949	Gannon, S. (2010) (Carbon 14)-DPX-HGW86: Absorption, Distribution, Metabolism and Excretion in Male and Female Rats. Project Number: DUPONT/16995/OCR, 15864, 1017. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 318 p.
48119950	Malley, L. (2006) DPX-HGW86 Technical: Acute Oral Neurotoxicity Study in Rats. Project Number: DUPONT/16996/OCR, 15850, 1261. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 254 p.
48119951	Gannon, S. (2010) (Carbon 14)-DPX-HGW86: Disposition in Male and Female Rats During and After Multiple Dose Administration. Project Number: DUPONT/17399/OCR, 15954, 1017. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 148 p.
48119960	Luckett, E. (2010) DPX-HGW86 Technical: Chronic Toxicity 1-Year Feeding Study in Dogs.

MRID	Citation Reference
	Project Number: DUPONT/19180/OCR, 125/056, 17021. Unpublished study prepared by MPI Research, Inc. 1406 p.
48119966	Mukerji, P. (2009) DPX-HGW86 Technical: Subchronic Oral Neurotoxicity Study in Rats. Project Number: DUPONT/19186/OCR, 17635, 1264. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 250 p.
48119967	Barnett, J. (2011) DPX-HGW86 Technical: Oral (Diet) Two-Generation (One Litter per Generation) Reproduction Toxicity Study in Rats. Project Number: DUPONT/19187/OCR, AUV00033, 17615. Unpublished study prepared by Charles River Laboratories. 2303 p.
48119968	Munley, S. (2009) DPX-HGW86 Technical: Developmental Toxicity in Rats. Project Number: DUPONT/19188/OCR, 17635, 841. Unpublished study prepared by E.I. du Pont de Nemours and Company. 341 p.
48119969	Munley, S. (2009) DPX-HGW86 Technical: Developmental Toxicity Study in Rabbits. Project Number: DUPONT/19189/OCR, 17635, 843. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 347 p.
48119970	Lowe, C. (2009) Cyantraniliprole (DPX-HGW86) Technical: 28 Day Repeat Dermal Application Study in Rats. Project Number: DUPONT/21316/OCR, 25531, 17635. Unpublished study prepared by Eurofins/Product Safety Laboratories. 245 p.
48119971	Hoban, D. (2009) Cyantraniliprole (DPX-HGW86) Technical: 28-Day Immunotoxicity Feeding Study in Rats. Project Number: DUPONT/21467/OCR, 17635, 1545. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 107 p.
48119972	Hoban, D. (2009) Cyantraniliprole (DPX-HGW86) Technical: 28-Day Immunotoxicity Feeding Study in Mice. Project Number: DUPONT/21468, 17635, 1546. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 105 p.
48119973	MacKenzie, S. (2010) Cyantraniliprole (DPX-HGW86) Technical: Adrenal and Thyroid Mechanistic: 90-Day Feeding Study in Rats. Project Number: DUPONT/24319/OCR, 17615, 1406. Unpublished study prepared by Experimental Pathology Labs., Inc., North Carolina State University and E.I. du Pont de Nemours and Company, Inc. 194 p.
48119974	Clarke, J. (2009) IN-JSE76: In vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT Assay): Final Report. Project Number: DUPONT/24714/OCR, AC22CC/782/BTL, 515. Unpublished study prepared by Bioreliance. 52 p.
48119975	Gudi, R.; Rao, M. (2010) IN-JSE76: In vitro Mammalian Chromosome Aberration Test: Final Report. Project Number: DUPONT/24715/OCR, AC22CC/341/BTL, 544. Unpublished study prepared by BioReliance. 65 p.
48119976	Wagner, V.; VanDyke, M. (2009) IN-JSE76: Bacterial Reverse Mutation Assay: Final Report. Project Number: DUPONT/24716/OCR, AC22CC/503/BTL, 17562. Unpublished study prepared by Bioreliance. 75 p.
48119979	Snajdr, S. (2010) Cyantraniliprole (DPX-HGW86) Technical: In vitro Thyroid Peroxidase Inhibition. Project Number: DUPONT/27123/OCR, 18092, 1407. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 24 p.
48119980	Wagner, V.; VanDyke, M. (2009) Cyantraniliprole (DPX-HGW86): Bacterial Reverse Mutation Assay: Final Report. Project Number: DUPONT/27160/OCR, AC25MV/503/BTL, 18255. Unpublished study prepared by Bioreliance. 76 p.
48119982	Wagner, V.; VanDyke, M. (2009) IN-N5M09: Bacterial Reverse Mutation Assay. Project

MRID	Citation Reference
	Number: DUPONT/28800/OCR, AC29WT/503/BTL, 18500. Unpublished study prepared by Bioreliance. 64 p.
48119983	Anand, S. (2010) IN-JSE76: Repeated-Dose Oral Toxicity 28-Day Feeding Study in Rats. Project Number: DUPONT/28842/OCR, 18499, 880. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 305 p.
48119985	MacKenzie, S. (2010) Cyantraniliprole (DPX-HGW86) Technical: Adrenal Mechanistic Study 90-Day Feeding Study in Mice. Project Number: DUPONT/29405/OCR, 18801, 1406. Unpublished study prepared by E.I. du Pont de Nemours and Company, Experimental Pathology Labs., Inc., and North Carolina State University. 117 p.
48120209	Fasano, W. (2008) Cyantraniliprole (DPX-HGW86) 100 g/L OD: In vivo Dermal Absorption of Cyantraniliprole in the Rat. Project Number: DuPont/24503. Unpublished study prepared by Dupont Haskell Laboratory. 57 p.
48120210	Fasano, W. (2008) Cyantraniliprole (DPX-HGW86) 100 g/L OD: In vitro Kinetics of Cyantraniliprole in Rat and Human Skin. Project Number: DuPont/24504. Unpublished study prepared by Dupont Haskell Laboratory. 98 p.
48120313	Fasano, W. (2009) Cyantraniliprole (DPX-HGW86) 200 g/Liter SC: In Vivo Dermal Absorption of Cyantraniliprole in the Rat. Project Number: DuPont/24501. Unpublished study prepared by Dupont Haskell Laboratory. 58 p.
48120314	Fasano, W. (2009) Cyantraniliprole (DPX-HGW86) 200 g/Liter SC: In vitro Kinetics of Cyantraniliprole in the Rat and Human Skin. Project Number: DuPont/24502. Unpublished study prepared by Dupont Haskell Laboratory. 100 p.
48120412	Fasano, W. (2009) Cyantraniliprole (DPX-HGW86) 100 g/L SE: In vitro Kinetics of Cyantraniliprole in Rat and Human Skin. Project Number: DUPONT/27073/OCR, 18089, 1377. Unpublished study prepared by E.I. du Pont de Nemours and Company. 97 p.
48120413	Fasano, W. (2009) Cyantraniliprole (DPX-HGW86) 100 g/L SE: In vivo Dermal Absorption of Cyantraniliprole in the Rat. Project Number: DUPONT/27074/OCR, 18089, 1378. Unpublished study prepared by E.I. du Pont de Nemours and Company. 55 p.
48122577	Craig, L. (2011) Cyantraniliprole Technical (DPX-HGW86 Commercial Batch -412): Combined Chronic Toxicity/Oncogenicity Study 2-Year Feeding Study in Rats. Project Number: DUPONT/26842, 125/101, 18029. Unpublished study prepared by MPI Research, Inc. 4672 p.
48122578	Craig, L. (2011) Cyantraniliprole (DPX-HGW86) Technical: Oncogenicity Study 18-Month Feeding Study in Mice. Project Number: DUPONT/26843, 125/100, 18029. Unpublished study prepared by MPI Research, Inc. 2549 p.
48122580	Wagner, V.; Jois, M. (2010) IN-PLT97: Bacterial Reverse Mutation Assay: Amended Final Report. Project Number: DUPONT/30552, AD08NK/503/BTL, 19129. Unpublished study prepared by Bioreliance. 70 p.
48122581	Madraymootoo, W.; Jois, M. (2011) IN-PLT97: In vitro Mammalian Chromosome Aberration Test: Revised Final Report. Project Number: DUPONT/30551, AD08NK/341/BTL, 19129. Unpublished study prepared by Bioreliance. 66 p.
48122582	Clarke, J. (2010) IN-PLT97: In vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT Assay): Final Report. Project Number: DUPONT/30365, AD08NK/782/BTL, 19129. Unpublished study prepared by Bioreliance. 55 p.
48122587	Wagner III, V.; Jois, M. (2010) Cyantraniliprole (DPX-HGW86) Technical: Bacterial Reverse

MRID	Citation Reference
	Mutation Assay: Final Report. Project Number: DUPONT/30991, AD10PN/503/BTL, 19192. Unpublished study prepared by Bioreliance. 61 p.
48122588	Madraymootoo, W.; Jois, M. (2010) Cyantraniliprole (DPX-HGW86) Technical: In vitro Mammalian Chromosome Aberration Test: Final Report. Project Number: DUPONT/30990, AD10PN/341/BTL, 19192. Unpublished study prepared by Bioreliance. 64 p.
48122589	Clarke, J. (2010) Cyantraniliprole (DPX-HGW86) Technical: In vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT Assay): Final Report . Project Number: DUPONT/30992, AD10PN/782/BTL, 19192. Unpublished study prepared by Bioreliance. 54 p.
48208424	Wagner, V.; VanDyke, M. (2009) Cyantraniliprole (DPX-HGW86) Technical: Bacterial Reverse Mutation Assay. Project Number: DuPont/27900, AC25SL/503/BTL, 18256. Unpublished study prepared by Bioreliance. 78 p.
48208425	Gudi, R. ; Rao, M. (2009) Cyantraniliprole (DPX-HGW86) Technical: In vitro Mammalian Chromosome Aberration Test. Project Number: DUPONT/27559, AC25MV/341/BTL, 18255. Unpublished study prepared by Bioreliance. 64 p.
48208426	Gudi, R. ; Rao, M. (2009) Cyantraniliprole (DPX-HGW86) Technical: In vitro Mammalian Chromosome Aberration Test. Project Number: DUPONT/27901, AC25SL/341/BTL, 18256. Unpublished study prepared by Bioreliance. 65 p.
48208427	Luckett, E. ; Mawn, M. (2010) DPX-HGW86 Technical: Chronic Toxicity 1-Year Feeding Study in Dogs. Project Number: DUPONT/19180, 125/056, 17021. Unpublished study prepared by E.I. du Pont de Nemours and Company and MPI Research, Inc. 18 p. *(Supplemental study of MRID 48119960)*
48208443	Stankowski, L. (2011) Cyantraniliprole (DPX-HGW86) Technical: CHO/HPRT Forward Mutation Assay with Duplicate Cultures. Project Number: DuPont/31372, 8236883, 515. Unpublished study prepared by Covance Laboratories, Inc. 68 p.
48208444	Donner, M. (2011) Cyantraniliprole (DPX-HGW86) Technical: Mouse Bone Marrow Micronucleus Test. Project Number: DuPont/31373, 18256, 572. Unpublished study prepared by E.I. du Pont de Nemours and Company, Inc. 65 p.
48208474	Mawn, M. (2011) IN-JSE76: Repeated-Dose Oral Toxicity 28-Day Feeding Study. Project Number: DUPONT/28842, 18499, 880. Unpublished study prepared by E.I. du Pont de Nemours and Company. 24 p. *(Supplemental report of MRID 48119983)*
48432412	Davies, D. (2011) Cyantraniliprole WG (A16971B) - In vitro Absorption through Human Epidermal Membranes using [Carbon 14]-Cyantraniliprole: Final Report. Project Number: TK0024294, JV2133, JV2133/REG. Unpublished study prepared by Dermal Technology Laboratory, Ltd. 63 p.
48432511	Runacres, S.; Harris, S. (2011) Cyantraniliprole/Thiamethoxam WG (A16901B) - In vivo Dermal Absorption in the Rat from WG Formulation A16901B: Final Report. Project Number: TK0027021, SGA/56. Unpublished study prepared by Quotient Bioresearch (Rushden) Limited. 70 p.
48432512	Davies, D. (2011) Cyantraniliprole/Thiamethoxam WG (A16901B) - In vitro Absorption through Human Epidermal Membranes using [14C]-Cyantraniliprole: Final Report. Project Number: TK0024297, JV2139/REG. Unpublished study prepared by Dermal Technology Laboratory, Ltd. 65 p.
48432513	Davies, D. (2011) Cyantraniliprole/Thiamethoxam WG (A16901B) - In vitro Absorption through Rat Epidermal Membranes using [Carbon 14]-Cyantraniliprole: Final Report. Project

MRID	Citation Reference
	Number: TK0024838, JV2140/REG. Unpublished study prepared by Dermal Technology Laboratory, Ltd. 61 p.
48663602	Sheung, P. (2011) Cyantraniliprole (DPX-HGW86) Technical: Four-week Inhalation Toxicity Study in Rats. Project Number: DUPONT/32967 19536 782. Unpublished study prepared by E.I. du Pont de Nemours and Company. 466p.
48894806	Bentley, K. ; DeLorme, M. (2012) Cyantraniliprole: Additional Information in Support of DuPont-32967, Four-week Inhalation Toxicity Study in Rats. Project Number: DUPONT/35858/OCR. Unpublished study prepared by DuPont Crop Protection. 11p. *(Supplemental report of MRID 48663602)*

Revised by US EPA (Primary reviewer: Whang Phang, PhD)

IIA 5.8/10

Report: Myhre, A. (2006); IN-F6L99: Bacterial reverse mutation test. DuPont Haskell Laboratories, Newark, Delaware, USA. Testing Laboratory Report No. DuPont-20597. November 15, 2006. MRID 46979931. Unpublished.

Guidelines: OPPTS 870-5100
EEC 2000/32/EC,
OECD 471
JMAFF 59 Nousan number 4200
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In a bacterial reverse mutation assay (MRID 46979931), IN-F6L99 (98.6% purity) was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and in *Escherichia coli* strain WP2 *uvr*A, with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). In the initial toxicity-mutation test (Tarial I), dose levels of 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 μg/plate were tested using standard plate incorporation methods. In the confirmatory mutagenicity test (Trail II), dose levels of 333, 667, 1000, 3333, and 5000 μg/plate were evaluated. The highest dose level was set based on the solubility of the test substance, and the limit dose (5000 μg/plate)for this test system. The test substance was adminitered to the test system as a solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 50 mg/mL.

Under the testing conditions, no toxicity was observed at any dose level and with any strain,. The number of revertants at all concentrations of the test substance was similar to concurrent controls in trials both with and without activation. Therefore, under the conditions of this study, IN-F6L99 was negative for mutagenic activity in both the non-activated and the S9-activated test systems.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for a bacterial reverse mutation assay (OPPTS 870-5100; OECD 471).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-F6L99 technical metabolite
   Lot/Batch #: F6L99-004
   Purity: 98.6%
   Description: Off-white solid
   CAS #: 500008-45-7
   Stability of test compound: Stable when held for 5 hours at room temperature
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)

Positive control, non activation:

Positive control	Dose (µg/plate)	Strain(s)
4-nitroquinoline-N-oxide	1.0	WP2 *uvr*A
acridine mutagen ICR-191	2.0	TA1537
sodium azide	2.0	TA100, TA1535
2-nitrofluorene	1.0	TA98

Positive control, activation:

Positive control	Dose (µg/plate)	Strain
benzo[a]pyrene	2.5	TA98
2-aminoanthracene	2.5	TA100, TA1535, TA1537
2-aminoanthracene	25	WP2 *uvr*A

3. Activation: Rat liver S9 from male Sprague-Dawley rats (500 mg/kg Aroclor 1254-induced)
   Lot number: Not provided
   Source: Moltox Inc., Boone, NC
   Protein content: 38.1 mg/mL
   Source: Not applicable
   Characterisation: Not applicable
   S9 mix composition
   Sodium phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM
   $MgCl_{2:}$ 8 mM
   S9: 10% (v/v)

4. Test organisms

The tester strains used, *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvr*A, were properly maintained and confirmed for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

Trial 1: IN-F6L99 was evaluated at concentrations of 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 μg / plate in duplicate in the presence and absence of S9 activation.

Trial 2: IN-F6L99 was evaluated at concentrations of 333, 667, 1000, 3333, and 5000 μg/ plate in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

September 20, 2006 to –October 09, 2006

2. Plate incorporation assay

This study consisted of 2 independent trials that assessed test substance mutagenicity. For the first trial, 2 replicates were plated for each tester strain in the presence and absence of the exogenous metabolic activation system at each test substance concentration. The second trial was conducted on triplicate plates. Positive and negative controls were included for each strain with and without activation. Treatments with the exogenous metabolic activation system were conducted by adding 0.1 mL of negative or positive control or test substance solution, 0.5 mL of metabolic activation system, and 0.1 mL of an overnight culture containing approximately 10^8 bacteria to approximately 2 mL of top agar (0.8% [w/v] agar and 0.5% [w/v] NaCl) containing 0.05 mM L-histidine, D-biotin, and L-tryptophan. These components were mixed and poured onto a minimal glucose agar plate. Treatments in the absence of the metabolic activation system were the same as those in the presence of the exogenous metabolic activation system with the exception that 0.5 mL of sterile buffer was used as a replacement for the volume of the exogenous metabolic activation system. After pouring onto the surface of minimal glucose agar plates, the top agar was allowed time to solidify, and the individually labeled plates were inverted and incubated at approximately 37°C for approximately 48 to 72 hours. Plates were refrigerated at approximately 4°C prior to evaluation and counting of revertant colonies.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Toxicity was scored relative to the concurrent negative control plates and recorded with the mean revertant count for the strain, condition and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter or entirely by hand.

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

A test substance was classified as positive when the mean number of revertants in any strain except TA1535 and TA1537 and at any test substance concentration was at least 2 times greater than the mean number of revertants in the concurrent negative control and occurred in a positive dose-response relationship. For strains TA1535 and TA1537, a mean number of revertants of at least 3 times greater than negative control was needed to be considered a positive response.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

IN-F6L99 was present at acceptable concentrations in the dosing solutions. IN-F6L99 was shown to be stable in the dosing solutions under the conditions of the study. IN-F6L99 was not found in the 0 mg/mL samples.

C. MUTATION ASSAYS

The test substance was soluble in DMSO at a maximum concentration of 50 mg/mL, and DMSO was determined to be an appropriate solvent based on the solubility of the test substance and compatibility with the target cells. The results of Trial I (toxicity-mutation test) and Trial II (confirmatory mutation assay) with and without S9 activation are presented in Tables 1 and 2, respectively..

No test substance-related toxicity was observed in either trial as indicated by no reduction in the background lawns and/or by a concentration-related decrease in mean revertants per plate relative to the controls. All positive controls exhibited more than a 3-fold increase in mean revertants over the respective mean of the negative controls. There was no concentration-related increase in the mean revertants per plate in any strain. No positive mutagenic responses were observed at any dose level or with any tester strain in either the absence or the presence of S9.

Table 1. Summary of average revertants/plate without activation

Compound	Conc. μg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-F6L99	0	15	27	85	117	11	7	6	8	35	28
	33.3	26	Ne	103	Ne	14	Ne	12	Ne	24	Ne
	66.7	20		94		11		8		41	
	100	21		90		13		13		36	
	333	14	20	106	111	7	12	8	6	32	32
	667	14	18	100	112	14	9	7	5	35	37
	1000	14	20	92	112	11	14	15	6	46	37
	3333	17	19	89	114	15	9	10	5	30	28
	5000	17	20	78	116	14	11	4	7	35	28
NAAZ	2.0	Ne		846	1005	748	638	Ne			
ICR-191	2.0	Ne						2186	1875	Ne	
2NF	1.0	147	137	Ne							
4NQ	1.0			Ne						677	702

Trial I – An average of 2 replicates per dose level; Data excerpted from Table 22 on page 43 of the study report.
Trial II – An average of 3 replicates per dose level; Data excerpted from Table 24 on page 44 of the study report.
ne = Not evaluated
2NF = 2-nitrofluorene; NAAZ = Sodium azide; ICR-191 = Acridine mutagen ICR-191; 4NQ= 4-nitroquinoline N-oxide
Ne= Not evaluated

Table 2
Summary of average revertants/plate with activation

Compound	Conc. μg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-F6L99	0	22	18	117	125	10	11	12	9	34	38
	33.3	21	Ne	135	Ne	11	Ne	10	Ne	35	Ne
	66.7	29		124		12		7		40	
	100	26		122		12		8		40	
	333	20	23	117	128	9	9	7	6	38	40
	667	28	22	119	124	7	9	8	12	40	36
	1000	30	23	125	147	10	15	8	7	47	36
	3333	17	25	123	123	9	17	7	6	35	36
	5000	20	20	124	140	12	9	13	8	39	37
2AA	2.5	Ne		3710	3679	256	238	252	390	Ne	
	25	Ne								367	319
B[a]P	2.5	341	351	Ne							

Trial I – An average of 2 replicates/dose level; Data excerpted from Table 23 on page 43 of the study report.
Trial II – An average of 3 replicates/dose level; Data excerpted from Table 25 on page 44 of the study report.
2AA = 2-aminoantracene; B(a)P = Benzo[a]pyrene

III. CONCLUSION

IN-F6L99 was negative for mutagenic activity in the non-activated and S9-activated test systems in the bacterial reverse mutation assay.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.1 Oral 14 day toxicity

IIA 5.3.1/04

Report: Nabb, D.L. (2010); Cyantraniliprole (DPX-HGW86) technical: Repeated-dose oral toxicity 2-week gavage study in rats with metabolism and genetic toxicology. DuPont Haskell Laboratories, Newark, Delaware, USA; Experimental Pathology Laboratories, Inc., Herndon, Virginia, USA. Laboratory Report No**.:** DuPont-13430. Revision No. 1. March 2, 2010. MRID 48119938. Unpublished.

Guidelines: **None Guideline (**In house method)
Deviations: Not applicable

GLP: No. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 14-day study (MRID 48119938), cyantraniliprole was administered by gavage to male and female Crl:CD®(SD) IGS BR rats at doses of 0 (5 males, 5 females), 25 (23 males, 5 females), 300 (23 males, 5 females), and 1000 mg/kg bw/day (8 males, 5 females). For each dose, the first five males and females were designated for evaluation of subchronic toxicity and *in vivo* micronucleus studies (main study group). At the 25, 300, and 1000 mg/kg bw dose levels, the remaining males were designated for pharmacokinetic evaluation (metabolism group). A separate group of five males and five females each received a single dose of 2000 mg/kg bw for evaluation of genetic toxicology (genetic toxicology group).

Parameters evaluated in the main study group included body weight, clinical signs, clinical chemistry, hematology, urinalysis, histopathology, organ weights, hepatic biochemistry (cytochrome P450, β-oxidation), thyroid hormone levels, and genetic toxicology. Parameters evaluated in the metabolism group included body weight, clinical signs, and pharmacokinetics. Parameters evaluated in the genetic toxicology group included body weight, clinical signs, and micronuclei in peripheral blood.

The results showed no adverse test substance-related effects on clinical pathology (hematology, clinical chemistry, and urinalysis), gross examination, histopathology, and organ weights. Increases in relative liver and adrenal weight were observed in the 1000 mg/kg bw/day females. As there was no histomorphologic correlate detected, these organ weight changes were considered test substance related but not adverse. There were no significant effects on TSH, T_3, or T_4 measurements at any dose level.

In the toxicokinetic assessment, the area under the plasma concentration *versus* time curve (AUC) was not proportional to the dose of cyantraniliprole at all time points. The half-life was estimated to be 3.84, 6.41, and 5.44 hours for the 25, 300, and 1000 mg/kg bw/day dose groups, respectively. The time of maximum concentration (T_{max}) was 2.00, 2.33, and 1.67 hours in the 25, 300, and 1000 mg/kg bw/day groups, respectively. The maximum concentration (C_{max}) was 15.53, 7.80, and 6.99 in the 25, 300, and 1000 mg/kg bw/day groups, respectively. Based on the

estimated half-life for cyantraniliprole, it was expected that there would be rapid clearance of the parent compound after 2 weeks of dosing. The concentration of cyantraniliprole in the perirenal fat was 0.016 and 0.062 µg/mL 24 hours after the final dose on test day 14 in the 25 and 300 mg/kg bw/day dose groups, respectively.

Total cytochrome P450 content in male or female rats was minimally elevated, but not statistically significant, at 1000 mg/kg bw/day. In male rats, cyantraniliprole was an inducer of cytochrome P450 isozyme CYP1A1 and CYP2B1. In female rats, cyantraniliprole was an inducer of cytochrome P450 isozyme CYP2B1. This enzyme induction is considered to be test substance-related but not adverse.

The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg bw/day for male and female rats, the highest dose tested. The NOAEL is based on lack of adverse effects at any dose tested.

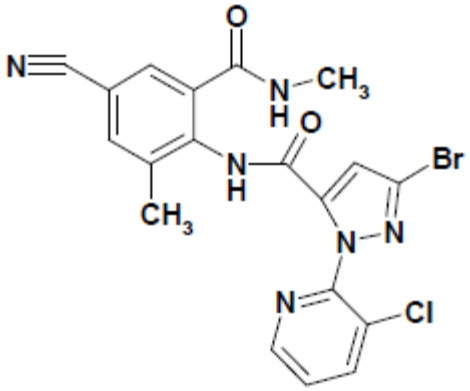
This study is classified as reliable (acceptable/non-guideline) and it provides useful information on the toxicity of cyantraniliprole with a 2-week treatment period.

I. MATERIALS AND METHODS

A. MATERIALS

1 Test material: Cyantraniliprole technical
.

3-Bromo-*N*-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxamide (Alternative IUPAC name)



Lot/Batch #:	HGW86-014
Purity:	~100%
Description:	White or yellow solid
CAS #	736994-63-1

	Stability of test compound:	Test substance stability was tested. Cyantraniliprole appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.
2.	Vehicle and/or positive control:	Polyethylene glycol (PEG400)
3	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)IGS BR
	Age at dosing:	6.5 weeks
	Weight at dosing:	181.0–245.7 g for males; 130.5–180.4 g for females
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	At least 3 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*.
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not reported
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   14-July-2003 to 04-August-2003

2. Animal assignment and treatment

   Cyantraniliprole was administered by gavage to male and female Crl:CD®(SD) IGS BR rats at doses of 0 (5 male, 5 female), 25 (23 male, 5 female), 300 (23 male, 5 female), and 1000 mg/kg bw per day (8 male, 5 female) for 14 days (Table 1). A separate group of five males and five females each received a single 2000 mg/kg bw dose for evaluation of genetic toxicology. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex.

Table 1. Study Design

mg/kgbw/day	No/Group		No of animals in Main Study[a]	Pharmacokinetic Evaluation	
	Males	Females		Serial Blood samples	Tissue collection
0	5	5	5/sex	--	--
25	23	5	First 5/sex	Next 3 males only	Remaining 15 (3/time point, males only)
300	23	5	First5 /sex	Next 3 males only	Remaining 15 (3/time point, males only)
1000	8 [c]	5	First 5/sex	Next 3 males only	--
2000	5	5	Single dose for genotoxici potential evaluation at the limit dose.		
20	5	5	Positive control for genotoxic potential evaluation, 20 mg/kg bw cyclophosphamide		

[a]: Main study animals were dosed for 14 consecutive days for subchronic toxicity and *in vivo* micronucleus evaluation.
Data excerpted from page 13 of the report.

3. Dose preparation and analysis

The test substance was suspended in polyethylene glycol (PEG400). The dose suspension remaining after dosing was collected once during the dosing period (*e.g.*, on test day 0) and stored frozen at -20°C for possible analytical analysis, but were not analyzed.

4. Statistics: Statistical methods used in this study are presented in Table 2.

Table 2. Statistical Methods

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Clinical pathology[a] Organ weight	Levene's test and Shapiro-Wilk test[b]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test
Plasma concentration Fat concentration Pharmacokinetic parameters β-Oxidation Cytochrome P450 Hormone levels	None	Descriptive statistics (*e.g.*, mean, standard deviation)	

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.10, 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported aa >1.083, 1.083 was used for any calculations performed with that data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS

1. Observations

Animals were observed twice daily for mortality and morbidity. Animals in the main study group were examined for clinical signs of toxicity at least once at weighing and 2 to 8 hours after each dosing. Animals in the metabolism group were examined for clinical signs on test days 15, 17, and 21 at weighing. Animals in the genetic toxicology group were examined for clinical signs at least once per hour for 4 hours post dosing.

2. Body weights

Animals in the main study and metabolism groups were weighed daily. Animals in the genetic toxicology group were weighed on test days 0, 12, 13, and 14.

3. Clinical pathology (hematology, clinical chemistry, urinalysis)

Blood and urine samples were collected from animals in the main study group approximately 14 days after initiation of the study. At sacrifice blood and bone marrow were collected. Hemotology, clinical chemistry, coagulation, and urine analysis were all preformed on the samples. Bone marrow smears were prepared at the final sacrifice from all main study animals, but experimental findings did not warrant analysis and samples were discarded. The following parameters were determined:

Hematology and coagulation

red blood cell count	ed cell distribution width
hemoglobin	absolute reticulocyte count
hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular(cell)hemoglobin concentration	
microscopic blood smear examination	
prothrombin time activated partial	thromboplastin time

Clinical chemistry parameters

aspartate aminotransferase	total protein
alanine aminotransferase	albumin
sorbitol dehydrogenase	globulin
alkaline phosphatase	calcium
total bilirubin	inorganic phosphorus
urea nitrogen	sodium
creatinine	potassium
cholesterol	chloride
triglycerides	creatine kinase
glucose	

UrinaUlysis

Quality	glucose	osmolality	urobilinogen
color	ketone	pH	protein
clarity	bilirubin	volume	blood

4. Pharmacokinectic Evalusitons

a. Blood Collection

Serial blood collection of approximately 0.25 mL of blood/animal/time point was taken on test day 14 from 3 rats/dose group via tail vein prior to dosing and then at 15 and 30 minutes, and 1, 2, 4, 8, 12, and 24 hours post-dosing on test day 14. Plasma was analyzed for parent compound.

b. Tissue/Sample Collection

Perirenal fat (approximately 1 gram), liver, and kidney were collected after sacrifice from 3 male rats/dose (low-and mid-dose groups, only) on test day 14 prior to dosing (time 0), and 6, 24, 72, and 168 hours after the final dose was administered on day 14. Blood samples were collected via the descending *vena cava.*

5. Biochemistry/mechanistic parameters

Blood was collected at necropsy from the inferior vena cava of the animals from each group designated for biochemical/hormonal evaluation. Serum was prepared and stored frozen until analysed for T_4, T_3, and TSH concentrations. Liver tissues were retained and processed for hepatic biochemical evaluations (beta-oxidation activity, total and specific cytochrome P450 content). The protein content of the microsomes was determined before and after analysis by the Biorad method, except for the microsomal samples used for specific cytochrome P450 analyses, which were only determined prior to analysis.

6. Micronucleus evaluation

Blood samples for the *in vivo* micronucleus (MN) analysis were collected from all main study and vehicle control animals, genetic toxicology animals, and positive control animals on test day 14 from the orbital sinus. The blood was prepared using the In Vitro MicroFlow Plus® Rat Micronucleus assay kit and fixed in ultra cold methanol and stored at < -65°C until processed. At least 15000 reticulocytes were analyzed per samples for micronuclei and bone marrow toxicity from samples collected from the control, 1000 mg/kg bw/day, and single-dose 2000 mg/kg bw/day groups.

7. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all animals sacrificed by design. Organs of main study rats that were weighed are listed in Table 2. Organ weight/final body weight ratios were calculated. Tissues collected from animals receiving the highest dose (1000 mg/kg bw/day) and control (0 mg/kg bw/day) were processed to slides and evaluated microscopically (Table 2).

Table 2. Organs/tissues collected for pathological examination		
Organ	**Organs weighed**	**Microscopic/histopathologic evaluation conducted[a]**
Liver	X	X
Kidneys	X	X
Thyroid gland	X	X
Adrenal glands	X	X
Brain	X	X
Esophagus		X
Stomach		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Lungs		X
Trachea		X
Nose		X
Heart		X
Aorta		X
Spleen		X
Thymus		X
Mandibular lymph node		X
Mesenteric lymph node		X
Pituitary gland		X
Parathyroid glands		X
Spinal cord		X
Skeletal muscle (thigh)		X
Femur/knee joint		X
Sternum		X
Testes		X
Epididymides		X
Prostate		X
Seminal vesicles		X
Ovaries		X
Uterus		X
Vagina		X
Skin		X
Mammary glands		X
Eyes		X

[a] Only tissues from animals in the highest dose and control groups were evaluated

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any dose groups in either males or females.

2. Mortality

   Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains. Statistically significant increases in body weight gain were observed at 25 and 300 mg/kg bw/day male groups over test days 8 to 9 and in the 300 mg/kg bw/day female group over test days 1 to 2 but were considered spurious.

C. CLINICAL PATHOLOGY

1. Hematology

   There were no statistically significant or biologically significant changes in hematology parameters.

2. Clinical chemistry

   Over all, there were no biologically significant changes in clinical chemistry parameters. A statistically significant lower globulin level in the high dose male animals was observed when compared to controls, but there were no changes in total protein or albumin. This change was not considered test substance related. Statistically significant lower bilirubin values in the mid dose female animals was observed when compared to control animals but was not found in a dose dependent manner and not considered treatment related.

3. Urinalysis

   There were no changes in urinalysis parameters that were considered adverse or treatment-related in male or female rats. A statistically significant increase in urine pH in the male high dose group was observed compared to controls, but were still within the normal range (pH 6 to 8) and not considered adverse.

D. PHARMACOKINETIC EVALUATION

After 14 consecutive days of dosing, the highest plasma concentration at various dose levels was approximately 2 hours after dosing (Table 3); At 24 hours post dosing, the plasma concentrationss approached pre-dose value (0.049, 0.24, and 0.25 μg/mL in the 25, 300, and

1000 mg/kg bw/day dose groups, respectively). The half-life (T1/2) of cyantraniliprole was 3.84, 6.41, and 5.44 hours in the 25, 300, and 1000 mg/kg bw/day dose groups, respectively. The time of maximum concentration (T_{max}) was 2.00, 2.33, and 1.67 hours in the 25, 300, and 1000 mg/kg bw/day groups, respectively (Table 4). The maximum concentration (C_{max}) was 15.53, 7.80, and 6.99 in the 25, 300, and 1000 mg/kg bw/day groups, respectively. Based on the estimated half-life for cyantraniliprole, it was expected that there would be rapid clearance of the parent compound after 2 weeks of dosing. In addition, the area under the plasma concentration *versus* time curve (AUC) was not proportional to the dose of cyantraniliprole at all time points.

Table 3. Plasma concentrations of cyantraniliprole in males (µg/mL)

mg/kg/day	25	300	1000
Pre-dose	0.034±0.008	0.168±0.117	0.355±0.198
0.5 hr	2.224±3.015	-	3.591±3.026
1.0 hr.	10.370±NC	7.294±8.033	3.934±2.545
2 hrs	15.527±6.680	2.481±1.328	5.179±3.234
4 hrs	6.006±4.441	2.847±0.703	2.470±1.528
8 hrs	1.432±1.022	1.536±0.366	2.158±1.325
12 hrs	0.465±0.267	0.905±0.510	1.863±0.670
24 hrs	0.049±0.036	0.240±0.008	0.250±0.061

NC: not calculated. Data excerpted from page 29 of the report
-: No data

Table 4. Pharmacokinetic Parameters in Male Rats

mg/kg/day	25	300	1000
T_{max} (hr)	2.00	2.33	1.67
C_{max}	15.53±	7.80±7.52	6.99±0.15
$T_{1/2}$	3.84±1.90	6.41±2.59	5.44±1.74
AUC^{+}	59.67±20.97	37.04±8.56	46.63±12.28

Data excerpted from page 55 of the report. +: Ares under the curve: plasma concentration vs. time

The concentration of cyantraniliprole in the fat was approximately 0.8 µg/mL at 6 hours post dosing afer final dosing (day 14) for both 25 and 300 mg/kg/day dose groups. By 1 day post dosing the concentrations in the fat were 0.02 and 0.06 µg/mL for 25 and 300 mg/kg/day dose groups, respectively. The data demonstrated that very little of the administered cyantraniliprole was sequestered in fat.

E. HEPATIC BIOCHEMICAL EVALUATION

Cyantraniliprole did not induce hepatic β-oxidation, a measure of peroxisome proliferation, in male or female rats. Total cytochrome P450 content in male and female rats was minimally higher, but not statistically significant, at 1000 mg/kg bw/day. In male rats, cyantraniliprole was a mild inducer of CYP1A1 at the 300 and 1000 mg/kg bw/day dose

levels and CYP2B1 at the 1000 mg/kg bw/day dose level after 14 days of dosing. In female rats, cyantraniliprole induced CYP2B1 at all dose levels.

Table 5. Summary of hepatic beta-oxidation and cytochrome P450 in male rats

mg/kg/day	0	25	300	1000
Males				
ß-oxidation rate (nmole/min/mg protein)	16.84±2.65	12.12±4.77	10.57±3.79	12.32±3.34
Total P450 content (nmol/mg)	0.69±0.09	0.71±0.14	0.62±0.16	0.82±0.08
CYP1A1 content (net intensity)	7094±1665	18099±4372 (↑155%)	25186±12908 (↑255%)	29719±14530 (↑319%)
CYP2B1 content (net intensity)	666±236	1120±531 (↑68%)	781±537 (↑17%)	2598±297 (↑290%)
Females				
ß-oxidation rate (nmole/min/mg protein)	20.84±6.94	15.45±5.39	12.62±4.51	13.61±2.94
Total P450 content (nmol/mg)	0.53±0.08	0.57±0.08	0.60±0.10	0.63±0.08
CYP1A1 content (net intensity)	5626±471	5488±1168 (↓2%)	6657±1315 (↑18%)	7308±3581 (↑30%)
CYP2B1 content (net intensity)	215±193	1284±533 (↑497%)	1461±542 (↑580%)	2305±411 (↑972%)

Data excerpted from pages 56-57 of the report

F. THYROID HORMONAL EVALUATION

There were no significant effects in T_3, T_4, or TSH in male or female rats.

G. GENETIC TOXICOLOGY EVALUATION

The report states that the micronucleus results were negative for both male and female rats. However, no flow cytometric assessment data were presented in the report except a small table stating that at dose levels of 1000 and 2000 mg/kg bw, it was negative (page 130 of the study report). It should be noted that the available mouse bone marrow micronucleus assay on cyantraniliprole was negative (MRID 48208444).

H. SACRIFICE AND PATHOLOGY

1. Organ weight

No test substance-related changes in mean organ weights or organ weights relative to final body weight were apparent at any dietary concentration. Relative liver weight (%BW, % brain) in female rats was increased in the 1000 mg/kg bw/day dose group compared to controls, but no histomorphologic correlate was detected in the examined high dose female livers. Relative adrenal gland weight (%BW, % brain) in female rats

was increased in the 1000 mg/kg bw/day dose group compared to controls, but no histomorphologic correlate was detected in the examined high dose female adrenal glands. Thus, the liver and adrenal weight increases were considered test substance related but not adverse.

2. Gross pathology and histopathology

   No test substance-related gross lesions were observed at necropsy. There were no test substance-related microscopic findings.

III. CONCLUSION

With gavage dosing at dose levels of 0, 26 300 and 1000 mg/kg bw for 14 days, no adverse treatment-related effects were observed at any dose levels for male and female rats. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg bw/day, the highest dose tested. increases in the frequency of micronucleated PCEs were observed in peripheral blood of male or female rats at any dose level.

In the toxicokinetic assessment, the area under the plasma concentration *versus* time curve (AUC) was not proportional to the dose of cyantraniliprole at all time points. The half-life was estimated to be 3.84, 6.41, and 5.44 hours for the 25, 300, and 1000 mg/kg bw/day dose groups, respectively. The time of maximum concentration (T_{max}) was 2.00, 2.33, and 1.67 hours in the 25, 300, and 1000 mg/kg bw/day groups, respectively. The maximum concentration (C_{max}) was 15.53, 7.80, and 6.99 in the 25, 300, and 1000 mg/kg bw/day groups, respectively. Based on the estimated half-life for cyantraniliprole, it was expected that there would be rapid clearance of the parent compound after 2 weeks of dosing. The concentration of cyantraniliprole in the perirenal fat was 0.016 and 0.062 µg/mL 24 hours after the final dose on test day 14 in the 25 and 300 mg/kg bw/day dose groups, respectively.

Total cytochrome P450 content in male or female rats was minimally elevated, but not statistically significant, at 1000 mg/kg bw/day. In male rats, cyantraniliprole was an inducer of cytochrome P450 isozyme CYP1A1 and CYP2B1. In female rats, cyantraniliprole was an inducer of cytochrome P450 isozyme CYP2B1. This enzyme induction is considered to be test substance-related but not adverse.

This study is classified as reliable (acceptable/non-guideline) and it provides useful information on the toxicity of cyantraniliprole with a 2-week treatment period.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.1/02

Report: Carpenter, C. (2009b); DPX-HGW86 technical: Repeated dose oral toxicity 28-day feeding study in mice. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.**:** DuPont-15205. March 20, 2009. MRID 48119940. Unpublished.

Guidelines: OECD 407 (1995)
OPPTS 870.3050 (2000)
Deviations: Majority of the recommended clinical chemistry parameters were not determined. Only plasma total protein was measured in this study.

GLP: No Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 28-day feeding study (MRID 48119940), cyantraniliprole (92.7%; HWG86-085) was administered to male and female Crl:CD1®(ICR) mice (10 animals/sex/concentration) at a concentration of 0, 300, 1000, 3000, or 7000 ppm (males:0, 53, 175, 528, and 1261 mg/kg bw/day; females: 0, 63, 212, 664, and 1476 mg/kg bw/day). Parameters evaluated in all mice included body weight, body weight gain, food consumption, food efficiency, clinical signs, gross pathology, and organ weights. Biochemical parameters (liver cytochrome P450 and β-oxidation) were evaluated in the first 5 animals per sex per concentration. Clinical pathology (hematology and plasma total protein in all groups) and microscopic pathology (control and 7000 ppm groups) were evaluated in the remaining 5 animals per sex per concentration.

Under the conditions of this study, cyantraniliprole did not affect survival, clinical observations, body weights, food consumption, food efficiency, clinical pathology parameters (hematology, plasma total protein), gross pathology, or histopathology.

At 3000 ppm and above, increased liver weight parameters were observed in males and in females, but the increases were considered to be an adaptive response and not biologically adverse. Cyantraniliprole did not induce hepatic β-oxidation in male or female mice. In male mice, the hepatic total cytochrome P-450 content was significantly increased at dietary concentrations of 3000 and 7000 ppm. In female mice, total cytochrome P-450 content was significantly increased at dietary concentrations of 300, 1000, 3000, and 7000 ppm. At 7000 ppm, increased liver weight was accompanied by an increase in the incidence of minimal focal necrosis of the liver in males (3/5) relative to the controls (1/5). Histopathological changes were not observed in 50, 300, 1000 and 3000 ppm males or females. Since the increased in the liver weight and hepatic cytochrome P-450 at concentration levels below 7000 ppm were not associated with histopathological findings in the liver, the changes at these levels were considered as adaptive effects and not adverse.

The no-observed-adverse-effect level (NOAEL) for males was 3000 ppm (528 and 644 mg/kg bw/day for male and female respectively). The LOAEL was 7000 ppm (1261 and 1476 mg/kg

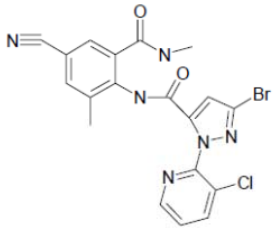
bw/day for males and females, respectively) based on increased incidence of minimal focal necrosis in the liver.

The study is classified as reliable (acceptable/non-guideline) and provides useful information for the understanding of short-term repeated dosing with cyantraniliprole (OPPTS 870.3050; OECD 407). Majority of the recommended clinical chemistry parameters were not determined; only plasma total protein was measured in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]phenyl]



Lot/Batch #:	HGW86-085
Purity:	92.7%
Description:	White solid
CAS #	736994-63-1
Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 7 days at room temperature, was distributed uniformly in the feed and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.

2. Vehicle and/or positive control: Untreated diet

3. Test animals

Species:	Mice
Strain:	Crl:CD®(ICR)
Age at dosing:	Approximately 49 days
Weight at dosing:	23.6–31.0 g for males; 21.1–26.0 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	12 days

	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4.	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not reported
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   13-July-2004 to 12-August-2004

2. Animal assignment and treatment

   Five groups of 10 animals/sex/concentration were administered a concentration of 0, 300, 1000, 3000, or 7000 ppm cyantraniliprole in feed daily for 29 (males) or 30 (females) days (Table 1). Dietary concentrations were chosen based on a previous two-week oral gavage study conducted in rats with this test substance where no effects on clinical signs, body weight, or clinical or microscopic pathology were observed at doses up to 1000 mg/kg bw/day (MRID 48119938). Liver weights were increased in female rats dosed 1000 mg/kg bw/day.

   Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study Design

No./sex group[a]	Conc. in diet (ppm)[b]	Mean daily intakes mg/kg bw	Mean daily intakes mg/kg bw
10	0 (control)	0	0
10	300	53	63
10	1000	175	212
10	3000	528	664
10	7000	1261	1476

Data excerpted from pages 12 (study design) and 40-41 (compound intakes) of the report.
[a]: The first 5 animals/sex/group were used to evaluate biochemical mechanistic endpoints. The last 5 animals/sex/group were used to in the main study for all other evaluations.
[b]: Weight/weight concentration of the test compound.

3. Diet preparation and analysis

The test substance was added directly to the rodent diet and thoroughly mixed for 3 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. The stability, homogeneity, and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC at the beginning and end of study. The test substance was at target concentrations (96% of nominal for the 300 ppm concentration; 96.4% of nominal for the 7000 ppm concentration), homogeneous (96.7 to 104% of nominal) throughout the feed and was stable (92.3 to 102.5% of nominal) for up to 21 days. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics: The method for statistical analyses are shown in Table 2, and they are appropriate.

Table 2. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Organ weight Mechanistic evaluation Clinical pathology[a]	Levene's test and Shapiro-Wilk test or Bartlett's test[b]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test
Survival Incidence of clinical observations	None	Cochran-Armitage test for trend[c]	

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with that bilirubin data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[c] If the incidence was not significant, but a significant lack of fit occurs, then Fisher's Exact test with a Bonferroni correction was used.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity. Detailed clinical observations were done weekly. General clinical observations were done on days detailed clinical observations were not done.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Biochemistry/mechanistic parameters

A portion of the liver from the first 5 animals per sex per group was homogenised and hepatic microsomes and peroxisomes prepared using differential centrifugation. The pellets were resuspended in homogenisation buffer and stored frozen until analysed for peroxisomal β-oxidation or total cytochrome P-450 content. The protein content of the microsomes was determined before and after analysis by using the Biorad kit.

5. Clinical pathology (hematology, clinical chemistry)

Blood was collected from all animals per sex per group approximately on test days 29 (males) and 30 (females) after initiation of the study. At sacrifice bone marrow was collected. The following hematology parameters were examined.

red blood cell count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
red cell distribution width
absolute reticulocyte count
platelet count
white blood cell count
differential white blood cell count
microscopic blood smear examination

Clinical chemistry: plasma total protein

6. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 3. Tissues collected from animals receiving the highest concentration (7000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically. Tissues from remaining animals were preserved for future analysis if needed.

Table 3. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Brain	X	X
Spleen	X	X
Heart	X	X
Liver	X	X
Kidneys	X	X
Esophagus		X
Adrenal gland	X	X
Duodenum		X
Jejunum		X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Gall bladder		X
Skin		X
Trachea		X
Nose		X
Larynx/pharynx		X
Thymus	X	X
Mandibular lymph node		X
Mesenteric lymph node		X
Bone marrow		X
Thyroid gland		X
Parathyroid glands		X
Eyes		X
Testes	X	X
Epididymides	X	X
Prostate		X
Seminal vesicles		X
Ovaries	X	X
Uterus	X	X
Mammary glands		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

[a] Only tissues from animals in the highest dose and control groups were evaluated

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

   Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no test substance-related effects on food consumption or food efficiency.

D. CLINICAL PATHOLOGY

1. Hematology

There were no adverse changes in hematology parameters in male or female mice. There was a slight drop in WBC in 7000 ppm males relative to the controls (0 ppm: 6.34±1.38; 7000 ppm: 5.32±1.55), but the decrease was not statistically significant and small. No changes in WBC were seen in females. It could not be considered as adverse.

2. Clinical chemistry

There were no adverse changes in clinical chemistry parameters measured in this study (plasma total protein) in male or female mice.

E. BIOCHEMISTRY/MECHANISTIC PARAMETERS

The data showed that cyantraniliprole did not induce hepatic β-oxidation in male or female mice (Table 4). In male mice, total hepatic cytochrome P-450 content was significantly increased at dietary concentrations of 3000 and 7000 ppm relative to the controls (↑54 and ↑55%, respectively). In female mice, total cytochrome P-450 content was significantly increased at dietary concentrations of 300, 1000, 3000, and 7000 ppm in comparison to the controls (68%, 98%, 123%, and 173%, respectively).

Table 4. Hepatic ß-oxidation activity & hepatic cytochrome P-450 content[a]

ppm	0	300	1000	3000	7000
Males					
ß-oxidation activity (nmol/min/mg protein)	17.9±6.6	17.4±1.9	17.1±4.0	17.1±3.0	15.9±1.2[b]
Cytochrome P-450 content (nmole/mg protein)	0.56±0.07	0.69±0.07	0.66±0.08	0.86±0.13* (↑54%)	0.87±0.07* (↑55%)
Females					
ß-oxidation activity (nmol/min/mg protein)	13.2±3.5	16.9±4.8	19.5±6.1	15.0±1.7	20.4±7.3[b]
Cytochrome P-450 content (nmole/mg protein)	0.40±0.13	0.67±0.07 (↑68%)	0.79±0.14* [b] (↑98%)	0.89±0.15* [b] (↑123%)	1.09±0.14* (↑173%)

Data excerpted from pages 96-97 of the report.
[a]: n= 5 unless specified. [b]: n=4. *: Statistically significant ($p<0.05$)

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Increased liver weights in males observed at 3000 ppm and above and in females at 300

ppm and above were considered to be an adaptive response to a xenobiotic and not biologically adverse (Table 5).

Table 5. Organ weights

ppm	0	300	1000	3000	7000
Males					
Absolute liver weight (g)	1.68±0.13	1.80±0.14	1.78±0.22	**2.08±0.18** * **(↑24 %)**	**2.06**±0.19* **(↑23%)**
Relative[a] liver weight (%)	5.01	5.32	5.45	**6.19*(↑24)**	**6.15*(↑23**
Liver to brain weight (%)	352	378	378	**449[b] (↑28)**	**460[b] (↑31)**
Females					
Absolute liver weight (g)	1.24±0.17	**1.43±0.13*** **(↑15%)**	**1.41±0.17 (↑14%)**	**1.51±0.17*** **(↑22%)**	**1.57±0.12*** **(↑27%)**
Relative[a] liver weight (%)	4.72	**5.18(↑10)**	**5.12(↑8)**	**5.53* (↑17)**	**5.72* (↑21)**
Liver to brain weight (%)	267	**296(↑11)**	**281 (↑5)**	**308* (↑15)**	**325* (↑22)**

[a] Relative weight is defined as the organ to body weight ratio.

* Significantly different from control by the Dunnett/Tamhane-Dunnett pairwise test criteria, $p < 0.05$.

Note: Bolded values were interpreted to be test-substance related increases, as compared to control values.

Data excerpted from pages 49-56 of the report.

2. Gross pathology and histopathology

There was an increase in the incidence of minimal focal necrosis of the liver in 7000 ppm males (3/5) relative to the controls (1/5). In 7000 ppm female group, one female was reported to have minimal focal necrosis of the liver while none was found in the control females. Histopathology findings were not observed in 50, 300, and 1000 ppm males and females. The liver lesion seen in 7000 ppm was considered as treatment–related and adverse effect. Additional histopathological lesions were not observed.

Table 6. Histopathology findings in the liver of treated mice

ppm	0	300	1000	3000	7000
Males					
Liver: focal necrosis, minimal	1/5	0/5	0/5	0/5	3/5
Inflammation, minimal	2/5	0/5	0/5	0/5	2/5
Fatty change, minimal	1/5	0/5	0/5	0/5	0/5
Females					
Liver: focal necrosis, minimal	0/5	0/5	0/5	0/5	1/5
Inflammation, minimal	4/5	0/5	0/5	0/5	3/5
Fatty change	No found				

Data excerpted from pages 74 & 85 of the study report.

III. CONCLUSION

Under the conditions of this 28-day oral toxicity study, cyantraniliprole produced an increase in liver weight in males at concentrations of 3000 ppm or above and in females at 300ppm or above. In addition, an associated increase in hepatic cytochrome P-450 was present in both males and females groups with liver weight increase. At 7000 ppm, there was an increase in the incidence of minimal focal necrosis of the liver in males (3/5) relative to the controls (1/5). This finding was treatment-related and adverse. There were not histopathological changes associated with the increase in liver weight and liver enzymes at 300, 1000, & 3000 ppm; the effects seen at these concentrations appeared to be an adaptive response. Therefore, the LOAEL was 7000 ppm

(1261 and 1476 mg/kg bw/day for male and female, respectively) based on increase incidence of minimal focal necrosis of the liver. The NOAEL was 3000 ppm (528 and 644 mg/kg bw/day for male and female respectively).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.1/01

Report: Carpenter, C. (2009a); DPX-HGW86 technical: Repeated dose oral toxicity 28-day feeding study in rats DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.**:** DuPont-15206. March 20, 2009. MRID 48119941. Unpublished.

Guidelines: OECD 407 (1995)
OPPTS 870.3050 (2000)
Deviations: None

GLP: No. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 28-day feeding study (MRID 48119941), cyantraniliprole (HGW86-085; 92.7%) was administered to male and female Crl:CD®(SD) rats (5 animals/sex/concentration) at dietary concentrations of 0, 600, 2000, 6000, or 20000 ppm (males: 0, 53, 175, 528, and 1776 mg/kg bw/day; females: 0, 62, 188, 595, and 1953 mg/kg bw/day). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, ophthalmology, gross pathology, histopathology, organ weights, hematology, coagulation, clinical chemistry, and hepatic biochemical measurements (cytochrome P450, β-oxidation, UDP-glucuronyltransferase).

The results showed that cyantraniliprole did not produce test substance-related deaths, clinical signs of toxicity, ophthalmological changes, or body weight and nutritional parameters channges. There were no treatment-related changes in clinical pathology parameters (hematology, clinical chemistry, coagulation, urinalysis) or on gross pathology.

Test substance-related increases were observed in liver weights and in the incidence of centrilobular hepatocellular hypertrophy. Mean liver weights were increased in 600 ppm or above males and in 2000 ppm or above females. The liver weight effect correlated with hepatocellular hypertrophy in male and female rats at similar dose levels. The liver effects were considered as an adaptive response. In male rats, cyantraniliprole was a mild inducer of hepatic UDP-glucuronyltransferase activity (range from ↑39% at low dose to ↑132% at high dose) while inducing only a minimal effect on cytochrome P-450 content. In female rats, cyantraniliprole caused a slight increase in total hepatic cytochrome P-450 content while inducing a somewhat lesser effect on hepatic UDP-glucuronyltransferase activity. These changes were considered not to be adverse. Cyantraniliprole did not induce hepatic β-oxidation, a measure of peroxisome proliferation, in male or female rats.

Mean thyroid weights were increased in male and female rats fed 600 ppm or above. The thyroid weight effect correlated with test substance-related follicular cell hypertrophy observed at similar dose levels in males and in females.

The no-observed-adverse-effect level (NOAEL) for male rats could not be established; LOAEL was 600 ppm (53 mg/kg bw/day)(lowest dose tested) based on treatment-related increase in the

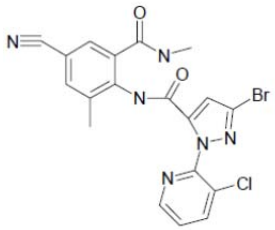
incidence of thyroid follicular hypertrophy. The NOAEL for female rats was 600 ppm (62 mg/kg bw/day) based on thyroid follicular hypertrophy observed in female rats fed 2000 ppm (188 mg/ kg bw/day) (LOAEL) and above.

This study is reliable (acceptable/non-guideline). The study yields useful information concern the toxicity of this chemical; however, a NOAEL can't be established. Therefore it does not meet the guideline requirement for a subchronic toxicity study (OPPTS 870.3050; OECD 407).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-085
Purity:	92.7%
Description:	White solid
CAS #	736994-63-1
Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 7 days at room temperature, was distributed uniformly in the feed and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.

2. Vehicle and/or positive control: Untreated diet
3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)
Age at dosing:	Approximately 42 days old
Weight at dosing:	149.5–183.3 g for males; 118.8–150.1 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	7 days

Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   22-June-2004 to 22-July-2004

2. Animal assignment and treatment

   Five groups of 5 animals/sex/concentration were administered concentrations of 0, 600, 2000, 6000, or 20000 ppm cyantraniliprole in feed daily for 28 days (for males; 29 for females). The dietary concentrations were selected based on the results from a previous 14-day oral toxicity study (via gavage) conducted in rats with this test substance (MRID 48119938). No effects on clinical signs, body weight, or clinical or microscopic pathology were observed at doses up to 1000 mg/kg bw/day. Liver weights were increased in female rats dosed at 300 or 1000 mg/kg bw/day.

   Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study Design

Conc. in diet (ppm)	No./sex/group	Mean daily intakes[a] (mg/kg bw/day)	
		Males	Females
600	5	53	62
2000	5	175	188
6000	5	528	595
20000	5	1776	1953

[a]: Data excerpted from pages 50 -51of the report.

3. Diet preparation and analysis

   The test substance was added directly to the rodent diet and thoroughly mixed for 3

minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. The stability, homogeneity, and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC at the beginning and end of study. The test substance was at target concentrations (100.3% of nominal for the 600 ppm concentration; 99.0% of nominal for the 20000 ppm concentration), homogeneous (99.0 to 102.0% of nominal) throughout the feed and was stable (96.5 to 104.3% of nominal) for up to 21 days. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Table 2. Statistics used in the study

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Organ weight Mechanistic evaluation Clinical pathology[a]	Levene's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test
Survival Incidence of clinical observations	None	Cochran-Armitage test for trend[c]	

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with that bilirubin data.

[b] If the Shapiro-Wilk test was not significant, but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[c] If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.

C. METHODS

1. Observations

   Animals were observed at least twice daily for mortality and morbidity. Detailed clinical observations were done weekly. General clinical observations were done on days detailed clinical observations were not done.

2. Body weights

   All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Clinical pathology (hematology, clinical chemistry, coagulation)

Blood was collected from the animals approximately 29 (males) or 30 (females) after initiation of the study. Hematology and clinical chemistry evaluations were performed on the samples. At sacrifice, blood for coagulation and bone marrow were collected. The following parameters were examined:

Hematology and coagulation

red blood cell count	red cell distribution width
hemoglobin	absolute reticulocyte count
hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular(cell)hemoglobin concentration	
microscopic blood smear examination	
prothrombin time activated partial	thromboplastin time

Clinical chemistry parameters

aspartate aminotransferase	total protein
alanine aminotransferase	albumin
sorbitol dehydrogenase	globulin
alkaline phosphatase	calcium
total bilirubin	inorganic phosphorus
urea nitrogen	sodium
creatinine	potassium
cholesterol	chloride
triglycerides	creatine kinase
glucose	

5. Urinalyses

The following parameters were examined:

quality	ketone
color	bilirubin
clarity	blood
volume	urobilinogen
osmolality	protein
pH	microscopic urine sediment examination
glucose	

6. Biochemistry/ mechanistic parameters

A portion of the liver from the rats was homogenised and hepatic peroxisomes and microsomes prepared using differential centrifugation. The pellets were resuspended in homogenisation buffer and stored frozen until analysed for UDP-GT, peroxisomal β-oxidation or total cytochrome P-450 content. The protein content of the microsomes was determined before and after analysis by the Biorad method.

7. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from all animals receiving the highest concentration (20000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically. Additionally, the liver and thyroid glands from all groups of male and female rats were processed to slides and evaluated microscopically.

Table 3. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Brain	X	X
Spleen	X	X
Heart	X	X
Liver	X	X
Kidneys	X	X
Esophagus		X
Adrenal gland	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Skin		X
Trachea		X
Nose		X
Larynx/pharynx		X
Thymus		X
Mandibular lymph node		X
Mesenteric lymph node		X
Bone marrow		X
Thyroid gland	X	X
Parathyroid glands		X
Eyes		X
Testes	X	X
Epididymides	X	X
Prostate		X
Seminal vesicles		X
Ovaries	X	X
Uterus	X	X
Mammary glands		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

[a] Only tissues from animals in the highest dose and control groups were evaluated with the exception of liver and thyroid which were evaluated in all groups.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No statistically significant or biologically significant changes in the incidence of clinical signs were observed for any dietary concentration in either males or females.

2. Mortality

   Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no test substance-related effects on food consumption or food efficiency.

D. CLINICAL PATHOLOGY

1. Hematology

There were no adverse changes in hematology parameters in male or female rats. However, increased degree of echinocytes and acanthocytes (changes in red cell shape) were observed in males and females fed 6000 or 20,000 ppm (Table 3a). Trace or few echinocytes and acanthocytes were often observed in control animals, and moderate echinocytes and acanthocytes are occasionally observed in control animals in the 90-day study (MRID 48119945). However, in this study, the number of animals with moderate or many acanthocytes and/or echinocytes was increased, compared to controls, in both males and females fed 6000 or 20,000 ppm. However, the incidence of echinocytes and acanthocyted was comparable among the treated groups and the control group. According to the study report, echinocytes and acanthocytes can be observed in the absence of changes in other red cell parameters. Therefore, these red cell shape changes were not necessarily related to changes in red cell mass parameters. Increased echinocytes and acanthocytes were considered to be treatment-related.

They were not considered adverse because the presence of increased incidence or degree of echinocytes was not expected to impact the functional capacity of red blood cells.

Table 3a. Incidence and grade/degree of echinocytes and acanthocytes

ppm	0	600	2000	6000	20000 ppm
Males (n=5)					
Echinocytes	**3**	**4**	**5**	**5**	**5**
trace	1	4	2	1	
few	2		3	4	
many					1
moderate					4
Acanthocytes	**3**	**5**	**5**	**5**	**5**
trace		3	3	2	
few	3	2	2	2	2
many					1
moderate				1	2
Females (n=5)					
Echinocytes	**5**	**5**	**5**	**5**	**5**
trace	3	3	1	1	1
few	2	2	2		1
many			2	2	3
moderate				2	
Acanthocytes	**3**	**5**	**5**	**5**	**5**
trace		3	3	2	
few	3	2	2	2	2
many					1
moderate				1	2

Data excerpted from the individual animal data on pages 162-165 of the study report.

2. Clinical chemistry

   There were no adverse changes in clinical chemistry parameters in male or female rats.

3. Coagulation

   There were no statistically significant or treatment-related changes in coagulation parameters in male or female rats.

4. Urinalysis

   There were no statistically significant changes in urinalysis parameters in male or female rats.

E. BIOCHEMISTRY/MECHANISTIC PARAMETERS

Cyantraniliprole did not induce hepatic β-oxidation, a measure of peroxisome proliferation, in male or female rats (Table 4). In male rats, cyantraniliprole induced increases in UDP-glucuronyltransferase activity at 600, 2000, 6000, and 20000 ppm (39%, 67%, 45%, and 132%, respectively) while inducing only a minimal increase in the cytochrome P-450 content at 20000 ppm (26%). In female rats, cyantraniliprole caused an increase (not

statistically significant) in total hepatic cytochrome P-450 content at 600, 2000, 6000, and 20000 ppm (39%, 34%, 52%, and 52%, respectively) while inducing somewhat similar increases (but not statistically significant) on hepatic UDP-glucuronyltransferase activity at the same dose levels (37%, 33%, 42%, and 38%, respectively).

Table 4 . Summary of hepatic beta-oxidation, cytochrome P450 content, and UDP-glucuronyltransferase activity

ppm	0	600	2000	6000	20000
Males					
ß-oxidation activity (nmol/mg protein)	11.3±1.1	9.2±1.8	10.9±2.4	9.4±3.5	9.0±1.1
Cytochrome P-450 content (nmol/mg protein)	1.03±0.32	1.05±0.58	1.05±0.58	1.05±0.16	1.30±0.35 (↑26%)
UDP-glucuronyl-transferase activity (nmol/mg protein)	9.6±2.3	13.3±5.5 (↑39%)	16.0±2.2* (↑67%)	23.5±3.4* (↑145%)	22.3±3.6* (↑132%)
Females					
ß-oxidation activity (nmol/mg protein)	15.2±3.1	13.0±3.1	15.4±4.0	17.2±2.2	15.0±2.5
Cytochrome P-450 content (nmol/mg protein)	0.50±0.08	0.68±0.24 (↑36%)	0.67±0.05[a] (↑34%)	0.76±0.08* (↑52%)	0.76±0.11* (↑52%)
UDP-glucuronyl-transferase activity (nmol/mg protein)	12.3±3.0	16.8±4.8 (↑37%)	16.3±3.8 (↑33%)	17.5±2.9 (↑42%)	17.0±2.9 (↑38%)

[a]: n=4

*: Statistically significant (p<05) by Dunn's test.

Data excerpted from pages 112-113 of the report.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

A test substance-related increase in mean liver weight parameters was observed in male rats fed 600 ppm or more of the test substance and in female rats fed 2000 ppm or more of the test substance (Table 5). Test substance-related increases in mean thyroid weight parameters were observed in male and female rats fed 600 ppm or more of the test substance.

Table 5. Selective absolute and relative organ weights

ppm	0	600	2000	6000	20000
Liver					
Males					
Absolute liver weight (g) (% control)	10.7±1.3	**11.8**±1.3	**12.1**±.1	**13.7**±1.6[a] **(↑29)**	**13.7**±1.5[a] **(↑29)**
Relative[a] liver weight (g) (% control)	3.3±0.2	3.5±0.2	**3.9**±0.2	**4.2**±0.2[b] **(↑26)**	**4.4**±0.4[b] **(↑31)**
Liver to brain weight (g) (% control)	543.4±75.6	**596.8**±57.1	**631.2**±52.0	**695.2**±66.6[a] **(↑28)**	**747.0**±93.1[a] **(↑37)**

Table 5. Selective absolute and relative organ weights					
ppm	**0**	**600**	**2000**	**6000**	**20000**
Female					
Absolute liver weight (g) (% control)	7.7 ±0.9	7.8±1.1	**9.1** ±1.5	**9.5** ± **0.8** **(↑24)**	**10.6**±1.8[a] **(↑38)**
Relative[a] liver weight (g) (% control)	3.7 ±0.2	4.0 ±0.2	**4.4** ±0.5	**4.5**±0.2[b] **(↑23)**	**5.2**±1.0[b] **(↑43)**
Liver to brain weight (g) (% control)	409.0 ±42.2	418.6±40.1	**501.0**±85.4	**525.4**±29.6[a] **(↑28)**	**564.7**±96.3[a] **(↑38)**
Thyroid					
Males:					
Absolute thyroid weight (g) (% control)	0.017 ±0.003	**0.021±0.003** **(↑24)**	**0.021±0.003** **(↑24)**	**0.024±0.005**[a] **(↑41)**	**0.020±0.004** **(↑18)**
Relative[a] thyroid weight (g) (% control)	0.005 ±0.001	**0.006 ±0.001** **(↑20)**	**0.007±0.001** **(↑40)**	**0.007±0.002**[a] **(↑40)**	**0.006±0.001** **(↑20)**
Thyroid to brain weight (g) (% control)	0.882 ±0.099	**1.083±0.16** **(↑23)**	**1.094±0.173** **(↑24)**	**1.222±0.280**[a] **(↑39)**	**1.104±0.143** **(↑25)**
Females:					
Absolute thyroid weight (g) (% control)	0.013 ±0.001	0.016±0.003 (↑23)	0.016±0.002 (↑23)	**0.018±0.002**[a] **(↑38)**	**0.022±0.003**[a,] **(↑69)**
Relative[a] thyroid weight (g) (% control)	0.006 ±0.000	**0.008±0.001** **(↑33)**	**0.008±0.001** **(↑33)**	**0.008±0.001** **(↑33)**	**0.011±0.002**[a] **(↑83)**
Thyroid to brain weight (g) (% control)	0.713 ±0.037	**0.838±0.122** **(↑18)**	**0.888±0.091** **(↑25)**	**0.971±0.089**[a] **(↑36)**	**1.162±0.188**[a] **(↑63)**

[a] Pair-wise test (Dunnett/Tamhane-Dunnett) significant Data excerpted from pages 68-74 of the report.

[b] Non-parametric comparision to control (Dunn's) significant

Note: Bolded values were interpreted to be test-substance related increases, as compared to control values.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. Increased incidence of centrilobular hepatocytes hypertrophy was observed in the livers of both treated males and females relative to the controls (0%, 20%, 60%, 100%, and 100% at 0, 600, 2000, 6000, and 20000 ppm males, respectively; and 0%, 20%, 80%, 60%, and 100% at 0, 600, 2000, 6000, and 20000 ppm females, respectively)(Table 6). The hepatocellular hypertrophy was graded as minimal (grade 1 out of 4), except for 2 females given 20000 ppm, where it was graded as mild (grade 2 out of 4). Microscopically, hepatocellular hypertrophy was characterized by an increased amount of finely granular eosinophilic cytoplasm within centrilobular hepatocytes. Hepatocellular hypertrophy and the associated increase in liver weights were considered a treatment-related pharmacological or adaptive response.

The incidence of thyroid follicular cell hypertrophy was increased in all treated males relative to the controls (0%, 20%, 60%, 60%, and 80% in 0, 600, 2000, 6000, and 20000 ppm males, respectively). In females the increase was seen in 2000, 6000, and 20000 ppm groups (20%, 20%, and 80% respectively) (Table 6). No thyroid follicular cell hypertrophy was found in the controls and 600 ppm animals. In all cases, the hypertrophy was graded as minimal (grade 1 out of 4). Follicular cell hypertrophy was

characterized by an increase in the columnar morphology of the thyroid follicular lining cells. Follicles were decreased in size, irregular in shape, and contained decreased amounts of normal pink colloid.

Table 6. Summary of liver and thyroid microscopic findings

ppm	0	600	2000	6000	20000
Males					
Liver Hypertrophy, Centrilobular	0/5	**1/5**	**3/5**	**5/5**	**5/5**
Thyroid Hypertrophy, Follicular cell	0/5	**1/5**	**3/5**	**3/5**	**4/5**
Females					
Liver Hypertrophy, Centrilobular	0/5	**1/5**	**4/5**	**3/5**	**5/5**
Thyroid Hypertrophy, Follicular cell	05	0/5	**1/5**	**1/5**	**4/5**

Note: Bolded values were interpreted to be test-substance related increases, as compared to control values.
Data excerpted from pages 91-111 of the report.

The registrant explained that the increase in the incidence and severity of thyroid follicular cell hypertrophy is indicative of altered thyroid gland homeostasis. In rats, a common cause of thyroid follicular cell hypertrophy is an increase in the rate of hepatic thyroxine (T_4) glucuronidation and subsequent biliary excretion. An increased rate of T_4 excretion results in lower T_4 blood levels which triggered an increase in the release of pituitary-derived thyroid stimulating hormone (TSH), resulting in thyroid follicular cell hypertrophy. Many inducers of hepatic cytochrome P-450 isoenzymes in the rat are known to cause secondary thyroid follicular cell hypertrophy by this mechanism. In this study, the presence of test substance-related hepatocellular hypertrophy in the males and females, increased cytochrome P450 content, and increased UDP-glucuronyltransferase activity demonstrates that hepatocellular enzyme systems have been induced and that T_4 excretion may have been secondarily increased. Due to differences in T_4 half-life, thyroglobulin binding, and the ease of UDP-glucuronyltransferase induction, rats are much more susceptible than humans to secondary thyroid follicular cell hypertrophy. The explanation is supported by the altered thyroid hormone (T3 & T4), TSH concentrations observed in the 90-day oral rat study (MRID 48119945) and the increased hepatic cytochrome P450 content and increased UDP-glucuronyl-transferase activity in the 28-day and 90-day studies.

III. CONCLUSION

Under the conditions of this study, cyantraniliprole did not produce test substance-related deaths, clinical signs of toxicity, ophthalmological changes, or body weight and nutritional parameters changes. There were no treatment-related changes in clinical pathology parameters (hematology, clinical chemistry, coagulation, urinalysis) or on gross pathology. However, it produced adaptive effects in the liver characterized by increased liver weight correlated with increased incidence of liver hypertrophy, and increased liver enzyme activity. It caused increase thyroid weight and corresponding thyroid follicular cell hypertrophy.

The no-observed-adverse-effect level (NOAEL) for male rats could not be established because at

600 ppm (53 mg/kg bw/day)(the lowest dose tested) treatment-related increase in the incidence of thyroid follicular hypertrophy was seen. The NOAEL for female rats was 600 ppm (62 mg/kg bw/day) based on thyroid follicular hypertrophy observed in female rats fed 2000 ppm (188 mg/ kg bw/day) (LOAEL) and above.

This study is reliable (acceptable/non-guideline). The study yields useful information concern the toxicity of this chemical; however, a NOAEL can't be established. Therefore it does not meet the guideline requirement for a subchronic toxicity study (OPPTS 870.3050; OECD 407).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.1/03

Report: Luckett, E.M. (2007); DPX-HGW86: 28-day oral palatability study in dogs. MPI Research, Mattawan, Michigan, USA. Laboratory Report No.: 125-052. DuPont Report No.: DuPont-15456. Fevruary 21, 2007. MRID 48119942. Unpublished.

Guidelines: Not applicable.

Deviations: None

GLP: No Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 28-day feeding study (MRID 48119942), cyantraniliprole (92.7%; HGW86-085) was administered in the diet to male and female beagle dogs (2 animals/sex/concentration) at concentrations of 0, 1000, 10000, and 40000 ppm (males: 0, 35, 311, and 1043 mg/kg bw/day; females: 0, 35, 335, and 1240 mg/kg bw/day). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, neurobehavioral observations, ophthalmoscopic observations, thyroid hormone parameters, gross pathology, organ weights, histopathology, and hepatic biochemical parameters (cytochrome P450).

Under the conditions of this study, cyantraniliprole did not produce treatment-related effects on survival, clinical or neurobehavioral findings, physical or ophthalmoscopic examinations, or in macroscopic pathology. Dose-related decreases in body weight, body weight gain were noted at all dietary concentrations. Corresponding decreases in food consumption and food efficiency were also found. Effects on clinical chemistry were observed; the effects included increased alkaline phosphatase activity, decreased cholesterol and albumin levels at $\geq$ 1000 ppm, and increased GGT at $\geq$10000 ppm.

Liver weights were increased in males at 10000 ppm and above and in all female dose groups, but were not associated with microscopic pathology except minimal hepatocyte apoptosis observed in one 40000 ppm female with correlative clinical pathology changes (increased cytotoxic liver enzyme activities). One 40000 ppm male dog had systemic arteritis. No additional microscopic pathology finding could be attributed to test article exposure.

Increases in hepatic cytochrome P450 (total and 2B1, 3A2, and 4A1/2/3 isozymes) were observed in all male and female dose groups. The study report presented thyroid hormones (TSH, total T_3, total T_4) measurements in 2 dogs each group. The data were variable, but it showed a reduction in T_3 at 10000 and 40000 ppm males and females. The level of T_4 also appeared to be lower in both high dose males and females than the control; the drop was slight and variable. The TSH level were comparable between treated and control dogs.

The no-observed-adverse-effect level (NOAEL) for male and female dogs was not determined for this study based on changes in body weight, food consumption, food efficiency, and clinical chemistry in dogs fed 1000 ppm (35 mg/kg bw/day) (lowest dose tested) and above, and microscopic pathology indicative of hepatocelluar injury in one female dog at 40000 ppm was also observed.

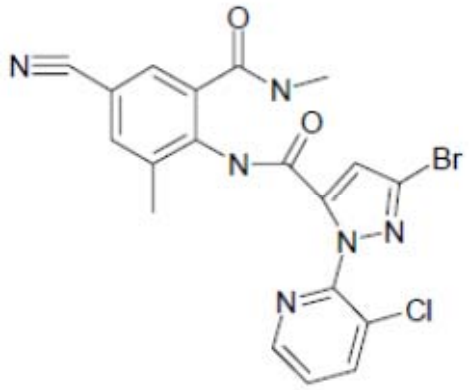
This study is considered useful for dose selection and provides information for evaluating the toxicity of this chemical. It is classified as acceptable/non-guideline.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical

1*H*-Pyrazole-5-carboxamide, 3-bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-cyano-2-methyl-6-[(methylamino)carbonyl]phenyl]- (inverted CAS name)



Lot/Batch #:	HGW86-085
Purity:	92.7%
Description:	White powder
CAS #	736994-63-1
Stability of test compound:	Analyses to confirm that the test material was stable, distributed uniformly and present in the feed at the target concentrations were not conducted for this study.

2. Vehicle and/or positive control: Untreated diet

3. Test animals

Species:	Dog
Strain:	Beagle
Age at dosing:	Approximately 5–6 months old
Weight at dosing:	7.25–7.93 kg for males; 5.37–6.16 kg for females
Source:	Covance Research Products, Kalamazoo, MI
Acclimation period:	At least one week

Diet:	PMI® Nutrition International, LLC Certified Canine LabDiet® (#5007). During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed individually in large animal runs with raised flooring in an environmentally controlled room.

4 Environmental conditions
.

Temperature:	64–84°F
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   18-March-2005 to 15-April-2005

2. Animal assignment and treatment

   Four groups of 2 animals/sex/concentration were administered concentrations of 0, 1000, 10000, and 40000 ppm cyantraniliprole in feed daily for 28 days. For females, animals were weighed prior to treatment and randomized into treatment groups using a standard, by weight, block randomization procedure. For males, according to the report, the animals were randomized using a simple randomization procedure into treatment groups based on testes volume.

Table1. Study design

Group no.	No./sex/ group	Conc. in diet (ppm)[a]	Males: Mean daily intakes[a] mg/kg bw	Females: Mean daily intakes[a] mg/kg bw
1	2	0 (control)	0 (control)	0 (control)
2	2	1000	35	35
3	2	10000	311	335
4	2	40000	1043	1240

[a]: Data excerpted from pages 95-96 of the report.

3. Diet preparation and analysis

   The required amount of test article was weighed and thoroughly ground with a portion of Lab Diet® using a mortar and pestle. This mixture was added additional Lab Diet® and mixed using a Hobart mixer. This resulting premix was added to additional Lab Diet® and blended for 10 minutes using a twin shell blender. The diet was divided into seven equal portions. Fresh test diets were prepared for each concentration weekly and

stored refrigerated. Homogeneity, stability, and concentration analyses were not conducted for this study.

4. Statistics: No statistical analyses were conducted since the number of test animal was only 2/sex/group.

C. METHODS

1. Clinical observations

   Animals were observed at least twice daily for mortality and morbidity and examined weekly for detailed clinical signs of toxicity. On occasion, clinical observations were recorded at unscheduled intervals.

2. Neurobehavioral observations

   Neurobehavioral observations were conducted weekly.

3. Body weight and body weight gain

   All animals were weighed once per week. Body weight change was calculated for each week and for the total study duration (weeks -1 to 4).

4. Food consumption, food efficiency, and daily intake

   Food consumption for each dog was measured and recorded daily during the study. Mean daily food consumption and compound consumption and mean food efficiency were calculated for each week and for the total study duration.

5. Ophthalmological examinations

   All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

6. Clinical pathology (haematology, clinical chemistry)

   Blood samples were collected from all animals at pretest and prior to termination. Hematology, clinical chemistry, and coagulation parameters were evaluated on the samples (Table 2).

Table 2. Clinical pathology parameters evaluated

Hematology	
Erythrocyte count	Leukocyte count (total and differential)
Hemoglobin	Reticulocytes (absolute and %)
Hematocrit	Platelet count
Mean corpuscular hemoglobin	Prothrombin time
Mean corpuscular volume	Activated partial thromboplastin time
Mean corpuscular haemoglobin concentration	

Table 2. Clinical pathology parameters evaluated	
Clinical chemistry	
Alkaline phosphatase	Total protein
Total bilirubin	Albumin
Aspartate aminotransferase	Globulin and A/G ratio
Alanine aminotransferase	Glucose
Gamma glutamyltransferase	Total cholesterol
Sorbitol dehydrogenase	Electrolytes (Na, K, Cl)
Urea nitrogen	Calcium
Creatinine	Phosphorus

7. Biochemistry/mechanistic parameters

A portion of serum was obtained from each blood sample collected for clinical chemistry evaluations (pre-test and at the end of the study) was separated into three aliquots (0.5 mL/aliquot) for evaluation of thyroxine (T_4), tri-iodothyronine (T_3), and thyroid stimulating hormone (TSH). Only results from the end of the study were reported.

A portion of the liver taken from each of these animals at necropsy was homogenised and hepatic microsomes prepared using differential centrifugation. Microsomal pellets were resuspended in homogenization buffer and stored frozen until analysed for total cytochrome P450 content. The protein content of the microsomes was determined before and after analysis by the Biorad method.

8. Sacrifice and pathology

At termination (Day 29), animals were sacrificed by administration of sodium pentobarbital solution and exsanguination. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight ratios were calculated. Tissues collected from animals receiving the highest dose (40000 ppm) and control (0 ppm) and all gross lesions were processed to slides and evaluated microscopically (Table 3).

Table 3. Organs/tissues collected for pathological examination			
Tissue	**Organs weighed**	**Collected and preserved**	**Microscopic/ histopathologic evaluation conducted[a]**
Adrenal gland	X	X	X
Aorta		X	X
Bone with bone marrow, femur		X	X
Bone with bone marrow, sternum		X	X
Bone with bone marrow, rib		X	X
Bone marrow smear[b]		X	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X
Epididymis	X	X	X

Table 3. Organs/tissues collected for pathological examination			
Tissue	**Organs weighed**	**Collected and preserved**	**Microscopic/ histopathologic evaluation conducted[a]**
Esophagus		X	X
Eye (with optic nerve)		X	X
Gallbladder		X	X
Heart	X	X	X
Kidney	X	X	X
Large intestine, cecum		X	X
Large intestine, colon		X	X
Large intestine, rectum		X	X
Liver	X	X	X
Lung		X	X
Lymph node, mandibular		X	X
Lymph node, mesenteric		X	X
Mammary gland (process females only)		X	X
Nerve, sciatic		X	X
Nictitans gland		X	X
Ovary	X	X	X
Pancreas		X	X
Parathyroid gland	X	X	X
Pituitary		X	X
Prostate		X	X
Salivary gland, mandibular		X	X
Skeletal muscle, biceps femoris		X	X
Skin		X	X
Small intestine, duodenum		X	X
Small intestine, ileum		X	X
Small intestine, jejunum		X	X
Spinal cord, cervical		X	X
Spinal cord, lumbar		X	X
Spinal cord, thoracic		X	X
Spleen	X	X	X
Stomach, cardia		X	X
Stomach, fundus		X	X
Stomach, pylorus		X	X
Testis	X	X	X
Thymus	X	X	X
Thyroid gland	X	X	X
Tongue		X	X
Trachea		X	X
Urinary bladder		X	X
Uterus with cervix	X	X	X
Vagina		X	X
Gross lesions		X	X

[a] Only tissues from animals in the highest dose and control groups and gross lesions in all groups were evaluated

[b] Bone marrow smears were prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

One 40000 ppm male presented signs of circling, head tilt, slight impaired limb strength, and inappetence over Weeks 3 to 4. The same dog was found to show vascullitis (arteritis) affecting the aorta and small to medium sized muscular arteries in the esophagus, heart, liver, lumber spinal cord meninges, and thyroid gland. An infarct in the thalamic region of the brain was seen in this dog, and it was attributed to inflammation and thrombosis of the artery supplying this area of the brain. The clinical signs (head tilt, circling, impaired limb strength) seen in this dog appeared to be a consequence of the vasculitis of thalamic region and not due to neurotoxic potential of cyantraniliprole.

2. Mortality

Cyantranilliprole did not affect the survival rate of the test dogs at any dose groups.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Relative to the controls dogs, the treated dogs at all dose levels showed a reduction in body weights (Table 4). At 40,000 ppm, one male and one female not only failed to gain any body weight, they showed a loss of its pre-test weight. There was a dose-related decrease in body weight gain in both males and females (Table 5). During the study, control animals gained 17 to 31% body weight. In comparison, animals at 1000 ppm gained 6 to 19%, but relative the controls, the body weight gain was decreased 63% in males and 40% in females. At 10000 ppm the body weight gained 0 to 7%, and the decreases in body weight gain were 81% and 77% in males and females, respectively). The deceases in body weight gains were dramatic in animals at 40000 ppm relative to the controls (179% and 115% in males and females respectively (Table 5).

Table 4. Mean body weights (kg) (n=2)

Day	0 ppm	1000 ppm	10000 ppm	40000 ppm
Males				
Week -1	7.4±0.2	7.8±0.1	7.9±0.1	7.5±0.3
Week 4	8.8±0.4	8.3±0.2 (↓6%)	8.1±0.3(↓8%)	6.4±1.9(↓27%)
Females				
Week -1	5.7±0.4	5.7±0.1	5.9±0.2	5.8±0.6
Week 4	7.1±0.1	6.5±0.5(↓8%)	6.2±0.3(↓13%)	5.6±1.0(↓21%)

Data excerpted from pages 71-72 of the report.

Table 5. Body weight gain (kg) (n=2)				
ppm	**0**	**1000**	**10000**	**40000**
Males				
Overall body weight gain (week -1 to 4)	1.40±0.23	0.52±0.09 (↓63%)	0.26±0.38 (↓81%)	-1.11±1.56 (↓179%)
Females				
Overall body weight gain (week -1 to 4)	1.35±0.45	0.81±0.42 (↓40%)	0.31±0.07 (↓77%)	-0.21±0.40 (↓115%)

Data excerpted from pages 77, and78 of the report. (% decrease relative to the controls)

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

When compared to the control mean, there was a dose-related decrease in food consumption and food efficiency in both male and female treated animals:

Table 6. Mean total food consumption/food efficiency (n=2)

Parameter	0 ppm	1000 ppm	10000 ppm	40000 ppm
Males				
Food consumption (g/an/day)[a] (week 1–4)	305.0±28.3	273.5±24.3 (↓10%)	249.1±54.3 (↓18%)	184.1±88.4 (↓40%)
Food efficiency (%) (week 1–4)	16.3	6.8 (↓58%)	3.2 (↓80%)	-32.5 (↓199%)
Females				
Food consumption (g/an/day) [a] (week 1–4)	230.5±26.9	215.8±22.0 (↓6%)	201.3±15.1 (↓13%)	172.2±30.3 (↓25%)
Food efficiency (%) (week 1–4)	20.7	13.0 (↓37%)	5.5 (↓74%)	-5.1 (↓124%)

[a] g/an/day = grams per animal per day. Data excerpted from pages 83-84 and 89-90 of the report. (% decrease relative to the control).

D. OPHTHALMOLOGICAL EXAMINATIONS

No test article-related ophthalmological effects were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Hematology

There were no biologically significant changes in any of haematological parameters. However, one male dog (#113) at 40000 ppm showed increases in leukocytes, neutrophils, and monocytes relative to the controls (Table 7). This increase appeared to be associated with Canine Juvenile Polyarteritis syndrome (CJPS).

Table 7. Summary of white blood cell counts. (n=2)

Dose group	Leukocytes (10^3/µL)	Neutrophils (10^3/µL)	Monocytes (10^3/µL)
0 ppm – male- #101 and 102	10.25	7.12	1.075
40000 ppm - male#113	40.9 (↑299%)	32.64 (↑358%)	3.80 (↑253%)

2. Clinical chemistry

Changes in the clinical chemistry parameters were observed in all male and female dose groups (Table 8). However, due to the number of dogs in each dose group (2 dogs/sex/grop), statistical analysis could not be conducted. Alkaline phosphatase activities were minimally to moderately increased at ≥1000 ppm (males and females). Albumin concentrations were mildly decreased at ≥1000 ppm (males and females). Cholesterol concentrations were moderately to severely decreased at ≥1000 ppm (males) and ≥10000 ppm (females). Gamma glutamyltransferase (GGT) activities were minimally increased at ≥10000 ppm (females only). Enzyme activities indicative of hepatocellular injury [aspartate and alanine aminotransferases (AST & ALT, respectively), sorbitol dehydrogenase] were increased in one 40000 ppm female.

Table 8. Clinical chemistry evaluations (n=2)

Parameter (unit)	0 ppm	1000 ppm	10000 ppm	40000 ppm
Males				
Alkaline Phosphatase (U/L)	88.0±7.1	189.5±70.0	305.0±97.6	595.0±472.4
GGT[a] (U/L)	3.0±0.0	5.5±0.7	4.0±0.0	3.5±0.7
AST[b] (U/L)	28.0±1.4	26.0±2.8	26.5±9.2	49.0±2.8
ALT[c] (U/L)	23.0±4.2	32.5±5.0	29.0±4.2	27.0±9.9
Sorbitol Dehydrogenase (U/L)	12.6±0.8	12.4±3.7	6.5±1.3	7.4±3.1
Total Protein (g/dL)	5.5±0.2	4.6±0.4	4.5±0.1	5.1±0.8
Albumin (g/dL)	3.0±0.1	2.0±0.2	2.0±0.1	1.6±0.1
Cholesterol (mg/dL)	167.5±27.6	100.0±60.8	56.5±20.5	121.0±103.2
Females				
Alkaline Phosphatase (U/L)	91.0±1.4	349.0±17.0	376.5±153.4	487.5±451.8
GGT[a] (U/L)	3.0±0.0	4.0±0.0	6.0±0.0	7.0±0.0
AST[b] (U/L)	34.0±2,8	34.0±.4	30.0±5.7	53.5±38.9
ALT[c] (U/L)	30.0±8.5	24.5±2.1	36.0±11.3	134.5±118.0
Sorbitol Dehydrogenase (U/L)	14.1±2.6	12.0±0.6	10.2±2.6	14.1±8.0
Total Protein (g/dL)	5.5±0.2	5.2±0.2	4.3±0.7	4.4±0.2
Albumin (g/dL)	2.9±0.2	2.2±0.2	1.8±00.4	2.0±0.4
Cholesterol (mg/dL)	135.0±22.6	146.0±4.2	68.5±61.5	43.5±26.2

[a] GGT = Gamma Glutamyltransferase. Data excepted from pages 107-112 of the report.
[b] AST = Aspartate aminotransferase [c] ALT = Alanine aminotransferase

F. BIOCHEMISTRY/MECHANISTIC PARAMETERS

The study report presented thyroid hormones (TSH, total T_3, total T_4) measurements in 2 dogs each group. The data were variable, but it showed a reduction in T_3 at 10000 and 40000 ppm males and females (Table 9). The level of T_4 also appeared to be lower in both high dose males and females than the control; the drop was slight and variable. Increases in hepatic cytochrome P450 (total P450 and isozymes 2B1, 3A2, and 4A1/2/3 isozymes) were observed in all male and female dose groups, but did not exhibit a dose-response (Table 10).

Table 9. Thyroid hormones in treated dogs

Males

	Dietary	TSH (ng/mL)			T3 (ng/dL)			T4 (µg/dL)		
	Conc (ppm)	pretest	Term	Change	Pretest	Term	Change	Pretest	Term	Change
101	0	0.273	0.046	-0.227	79.976	70.459	-9.517	1.490	0.453	-1.037
102	0	0.054	0.015	-0.039	63.272	62.048	-1.224	1.166	0.633	-0.533
105	1000	0.247	0.042	-0.205	80.368	48.252	-32.116	1.704	1.000	-0.704
106	1000	0.077	0.000	-0.077	61.695	0.000	-61.695	1.404	0.155	-1.249
109	10,000	0.204	0.000	-0.204	79.336	11.734	-67.602	1.307	0.312	-0.995
110	10,000	0.090	0.000	-0.090	62.552	26.508	-36.044	0.807	0.291	-0.516
113	40,000	0.096	0.176	0.080	59.129	12.954	-46.175	1.316	0.000	-1.316
114	40,000	0.000	0.000	0.000	60.734	0.000	-60.734	1.190	0.000	-1.190

Females

	Dietary	TSH (ng/mL)			T3 (ng/dL)			T4 (µg/dL)		
	Conc (ppm)	pretest	Term	Change	Pretest	Term	Change	Pretest	Term	Change
103	0	0.000	0.000	0.000	52.100	56.616	4.516	0.833	0.332	-0.501
104	0	0.032	0.000	-0.032	66.228	62.857	-3.371	1.555	0.623	-0.932
107	1000	0.000	0.338	0.338	61.456	58.846	-2.610	0.864	0.240	-0.624
108	1000	0.163	0.208	0.045	58.896	73.018	14.122	1.090	0.778	-0.312
111	10,000	0.000	0.000	0.000	51.160	35.342	-15.818	0.983	0.030	-0.953
112	10,000	0.408	0.327	-0.081	61.062	2.760	-58.302	1.520	0.115	-1.405
115	40,000	0.254	0.000	-0.254	60.940	22.213	-38.727	1.212	0.050	-1.162
116	40,000	0.000	0.000	0.000	52.941	26.317	-26.624	1.222	0.217	-1.005

Term = study termination

Data excerpted from pages 266 and 267 of the report.

Table 10. Hepatic cytochrome P450 (n=2)				
ppm	**Total content (nmol/mg)**	**2B1/2 content (net intensity)x1000**	**3A2 content (net intensity) x1000**	**4A1/2/3 content (net intensity)x1000**
Males				
0	0.52±0.10	8.5±2.8	17.0±9.4	169.0±109.6
1,000	1.53±0.35	57.2±10.2	45.3±14.1	256.7±17.4
10,000	1.57±0.05	30.2±6.0	56.8±15.7	267.4±14.3
40,000	1.62±NA	14.0±12.1	25.8±16.7	146.3±12.3
Females				
0	0.75±0.07	5.1±2.3	6.3±0.9	63.8±5.0
1,000	1.61±0.34	32.5±1.9	54.0±16.0	110.5±0.1
10,000	1.67±0.01	60.4±5.8	84.7±33.9	113.7±1.3
40,000	1.45±0.51	56.1±25.0	60.4±18.9	119.1±72.3

NA= not available Data excerpted from pages 264-265 of the report.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The values for liver weight parameters were higher than control in males at 10000 and 40000 ppm and females at 1000, 10000, and 40000 ppm (Table 9). The increased liver weights were not correlated with hepatocellular hypertrophy or adverse microscopic changes and were not considered adverse. No other test substance-related changes in mean organ weights or organ weights relative to final body weight were apparent at any dietary concentration.

Table 9. Organ weights (n=2)

Parameter	***0 ppm***	***1000 ppm***	***10000 ppm***	***40000 ppm***
Males				
Absolute liver weight (g)	284.3±4.2	287.2±151.1	**369.1±68.3**	**361.8±72.1**
Relative[a] liver weight (%)	3.7	3.9	**4.9**	**6.2**
Liver to brain weight (ratio)	4.2	4.1	**5.0**	**5.2**
Females				
Absolute liver weight (g)	195.8±7.6	**338.0±19.4**	**348.6±26.7**	**294.3±35.0**
Relative[a] liver weight (%)	3.3	**5.8**	**6.3**	**6.0**
Liver to brain weight (ratio)	2.8	**4.8**	**5.0**	**4.1**

[a] Relative weight is defined as the organ to body weight ratio. Data excerpted from pages 118 & 122 of the report.
Bolded values were interpreted to be test-substance related increases, as compared to control values.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. No test article-associated microscopic effects were observed in treated males or females, with the possible exception of minimal hepatocyte apoptosis in the liver of one female at 40000 ppm. In this female, subacute inflammation was also seen. The liver weight of this

female dog was also increased relative to the controls. As alluded to in Clinical Signs section, one male at 40000 ppm had systemic vasculitis (arteritis) affecting the aorta and small to medium-sized muscular arteries in the esophagus, heart, liver, mediastinum, lumbar spinal cord meninges, and thyroid gland. An infarct in the thalamic region of the brain was seen and was diagnosed to be secondary to the vasculitis. In addition, inflammation, degeneration, and/or necrosis secondary to the vasculitis were observed in various organs. The study author claimed that the microscopic findings in this dog were compatible with spontaneous canine juvenile polyarteritis syndrome (CJPS), as distinguished from test article-related arteritis, and these changes were not considered to be test article related. However, CJPS was thought to be mediated by the immune system, but cyantraniliprole did not show any effect on the immune system in two immunotoxicity studies in rats and mice (MRID 48119971 & MRID 48119972). At this time, the arteritis seen in this male was compound-related.

III. CONCLUSION

This study was intended as a dose-range finding study where only 2 dogs/sex/group were employed; the study also included measurements on thyroid hormones (T_3, & T_4), TSH, and hepatic cytochrome P450. Under the conditions of this study, cyantraniliprole produced decreased in body weights, body weight gains, food consumption, and food efficiency at all dose levels. Clinical chemistry parameter changes such as increased alkaline phosphatase and decreases in albumin and cholesterol levels were seen at $\geq$ 1000 ppm. At higher concentrations, GGT, AST, and ALT were also increased. However, cyantraniliprole did not affect thyroid hormones (T_3, & T_4), and TSH. It increased hepatic cytochrome P450 content and induced increases in the activity of the isozymes (2B1/2, 3A2, & 4A1/2/3) reported as increased intensity.

The no-observed-adverse-effect level (NOAEL) for male and female dogs was not determined for this study because decreases in body weight, body weight gains, food consumption, food efficiency, and changes in clinical chemistry in dogs at 1000 ppm (35 mg/kg bw/day) (lowest dose tested) and above and microscopic pathology indicative of hepatocelluar injury in one female dog at 40000 ppm.

This study is considered useful for dose selection and provides information for evaluating the toxicity of this chemical. It is classified as acceptable/non-guideline.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.2.2 Oral 90-day toxicity in the mouse

IIA 5.3.2/04

Report: MacKenzie, S.A. (2007); DPX-HGW86 technical: Subchronic toxicity 90-day feeding study in mice. DuPont Haskell Laboratories, Experimental Pathology Laboratories, Inc., Newark, Delaware, USA, Sterling, Virginia, USA. Laboratory Report No.: DuPont-16992. April 17, 2007. MRID 48119943

IIA 5.3.2/03

Report: Gannon, S.A. (2011b); DPX-HGW86 technical: Subchronic toxicity 90-day feeding study in mice. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory **Report No.:** DuPont-16993, Supplement No.1, Revision No. 1. April 6, 2011. MRID 48119944. Unpublished. (analysis of blood samples for metabolites)

Guidelines: OPPTS 870.3100 (1998),
OECD 408 (1998),
Commission Directive 2001/59/EC Part B.26 (2001),
MAFF 12 Nousan 8147 (2000)
Deviations: The majority of the recommended clinical chemistry parameters were not determined. Only total protein was measured.

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 90-day feeding study (MRID 48119943), Cyantraniliprole (93.4%; HGW86-141) was administered to male and female Crl:CD1®(ICR) mice (10 mice/sex/concentration) at concentrations of 0, 50, 300, 1000, and 7000 ppm (males: 0, 7, 47, 150, and 1092 mg/kg bw/day, respectively; females, 0, 10, 58, 204, and 1344 mg/kg bw/day, respectively) for at least 90 days (97 days for males; 98 days for females). Five satellite groups of Crl:CD1®(ICR) mice (5/sex/group) were also dosed at similar concentrations for 63 days. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, hematology, clinical chemistry, ophthalmology, organ weights, gross and microscopic pathology, and plasma concentration of test substance or metabolites. Blood was collected from the satellite mice on test day 63 (non-fasted) and analyzed for concentrations of parent compound and selected metabolites (Supplemental study, MRID 48119944).

The data showed that cyantraniliprole did not produce test substance-related deaths, clinical signs of toxicity, ophthalmological changes, or changes in body weights, food consumption and food efficiency. There were no treatment-related changes in clinical pathology parameters (haematology and plasma total protein). A test substance-related increase in liver weights was observed in males at 7000 ppm and in females at ≥1000 ppm. The increased liver weights correlated with hepatocellular hypertrophy at the same concentration levels. There was also an increase in the incidence of focal necrosis of the liver in the 7000 ppm females (4/10) and males (1/10). Focus of cellular alteration was also seen in one 7000 ppm male. At ≤ 1000 ppm, both the

liver weight increases and the hepatocellular hypertrophy were considered as an adaptive response associated with hepatic enzyme induction as demonstrated in other subchronic rat and mouse toxicity studies (MRID 48119945, MRID 48119940). In addition, microscopic examination revealed an increase in cortical microvesiculation in males fed ≥50 ppm. This finding was graded as minimal to mild, and it was considered to be a non-adverse change within normal physiological limits.

Metabolite analyses showed that IN-MLA84 was the most prevalent analyte present in male or female mice, followed by cyantraniliprole. Plasma values for all the evaluated metabolites were similar between males and females at comparable diet concentrations.

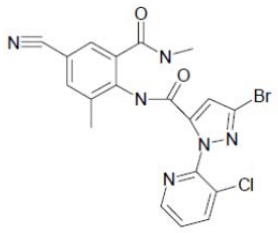
Based on the data, the NOAEL was 1000 ppm (150/204 mg/kg bw/day for males/females); LOAEL was 7000 ppm (1092/1344 mg/kg bw/day for males/females) based on increase in the incidence of focal necrosis of the liver and liver weight increase.

This study is reliable (acceptable/none-guideline), and the conclusions can be drawn from the results. The study meets most of the data requirements for a 90-day oral toxicity study in mice (OPPTS 870.3100; OECD 408 (1998)). However, the majority of the recommended clinical chemistry parameters were not determined. Only total protein was measured.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]phenyl]



Lot/batch No.:	HGW86-141
Purity:	93.4%
Description:	Solid, powder
CAS #	736994-63-1

Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 14 days at room temperature, was distributed uniformly in the feed and was present in the feed at targeted concentrations. Diets were prepared every week until stability data were confirmed. After confirmation of stability, diets were prepared every other week.
2. Vehicle and/or positive control:	Untreated diet
3. Test animals	
Species:	Mouse
Strain:	Crl:CD1®(ICR)
Age at dosing:	Approximately 50 days old
Weight at dosing:	25.4–33.9 g for males; 21.2–26.9 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	8 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions	
Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   03-June-2005 to 17-April-2007

2. Animal assignment and treatment

   Groups of 15 animals/sex/concentration were administered cyantraniliprole in the diet daily for at least 90 (15/sex/concentration) at dietary concentrations of 0, 50, 300, 1000, and 7000 ppm. Ten mice/sex/concentration were designated for evaluation of subchronic toxicity (Table 1). The remaining 5 mice/sex/concentration were designated for evaluation of plasma concentrations of parent compound and selected metabolites, following 63 days of dietary exposure. Concentrations were selected on the basis of previous 28-day feeding studies in mice and rats. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically

significant differences among group body weight means within a sex. The control group received untreated diet.

Table 1. Study design

Conc. in diet (ppm)[a]	No./sex/ Group [b]	Mean daily intakes [c] mg/kg bw	Mean daily intakes[b] mg/kg bw
0 (control)	15	0 (control)	0 (control)
50	15	7	10
300	15	47	58
1000	15	150	204
7000	15	1092	1344

[a]: weigh/weight concentration of test substance.
[b]: 5 animals/sex/group were treated for 63 days and evaluated for plasma concentrations of cyantraniliprole and its metabolites
[c]: The daily compound intake values were excerpted from pages 55 & 57 of the report, and rounded to whole numbers.

3. Diet preparation and analysis

The test substance was added to the rodent diet and thoroughly mixed for a period of time that, by experience, was adequate to ensure homogeneous distribution in the diet. Diets were prepared every week until stability data were confirmed. After confirmation of stability, diets were prepared every other week. All diets were refrigerated until used. The stability, homogeneity and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC. Samples for analysis were collected at the initial diet preparation, and near the middle and end of the study. Analytical results verified that all diets were within 10% of nominal at all evaluated times, were homogeneously mixed (coefficients of variance ≤8%), and were stable under relevant storage conditions. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics: The statistical methods employed in this study are summarized in Table 2, and they are appropriate.

Table 2. Statistical method employed

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, organ weight, clinical pathology[a]	Levene's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test
Survival, incidence of clinical observations, incidence of ophthalmology observations	None	Cochran-Armitage test for trend[c]	

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with those bilirubin data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with those data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[c] If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.

C. METHODS

1. Clinical observations

   Animals were observed at least twice daily for mortality and morbidity and once daily for acute clinical signs. All animals were examined at every weighing for detailed clinical signs of abnormal behaviour and appearance.

2. Body weights

   All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

   All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, urinalysis)

   Blood samples were collected from all animals on test days 97 (males) and 98 (females). At sacrifice blood and bone marrow were collected. The samples were evaluated for hematological and clinical chemistry parameters (Table 3).

Table 3. Clinical pathology parameters evaluated

Haematology	
Erythrocyte count	Red cell distribution width
Haemoglobin	Absolute reticulocyte count
Hematocrit	Platelet count
Mean corpuscular haemoglobin	White blood cell count
Mean corpuscular volume	Differential white blood cell count
Mean corpuscular haemoglobin concentration	Microscopic blood smear examination
Clinical chemistry	
Total protein	

6. Plasma concentration

   Blood (non-fasted) was collected from the satellite animals (5 animals/sex/concentrations) from the vena cava at sacrifice on test day 63, for determination of plasma concentration of test substance and selected metabolites. Samples were evaluated by liquid chromatography-mass spectroscopy (LC-MS).

7. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 4. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues were processed to slides and evaluated microscopically (Table 4) from all control and high dose mice and from mice that died prior to scheduled sacrifice. Gross lesions and suspected target tissues (liver, adrenal glands, testes, and epididymides) were also evaluated microscopically from all animals.

Table 4. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Liver	X	X
Gallbladder		X
Esophagus		X
Stomach		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Kidneys	X	X
Urinary bladder		X
Lungs		X
Trachea		X
Nose (four sections)		X
Larynx/pharynx		X
Heart	X	X
Aorta		X
Bone marrow		X
Thymus	X	X
Spleen	X	X
Mandibular lymph node		X
Mesenteric lymph node		X
Peyer's patches		X
Pituitary gland		X
Thyroid gland		X
Parathyroid glands		X
Adrenal glands	X	X
Brain (three sections)	X	X
Spinal cord (three levels)		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Testes	X	X
Epididymides	X	X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Prostate		X
Seminal vesicles		X
Ovaries	X	X
Uterus	X	X
Mammary glands		X
Skin		X
Eyes (including retina and optic nerve)		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

   Test substance-related mortality did not occur during the course of this study. One 1000 ppm male was found dead on day 62; the cause of death was undetermined. One 300 ppm female was found dead on day 72; the cause of death was renal amyloidosis.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no adverse test substance-related effects on body weights or body weight gains. Mean body weight on test day 91 in male and female 7000 ppm groups were 99% and 95% of controls, respectively. Mean overall (test days 0-91) body weight gains in male and female 7000 ppm groups was 104% and 87% of control, respectively. None of the changes were statistically significant.

Table 5. Mean body weights and body weight gains

Dose rate *ppm*	Body weights (g±SD)				Mean weight gain	
	Day 0	Day 7	Day 49	Day 91	gm	% of control
Males						
0	30.0±2.0	31.8±2.2	37.7±3.1	39.5±4.5	8.9±3.6	
50	29.5±1.6	32.2±1.7	38.5±2.8	41.7±3.1	11.6±2.5	130
300	28.8±1.9	31.1±2.2	37.5±3.3	39.4±4.7	10.6±3.4	119
1000	29.9±1.9	31.6±2.0	38.0±3.1	40.1±4.2	9.9±2.5	111
7000	29.3±1.8	31.3±2.5	36.4±2.9	39.1±3.3	9.3±1.9	104
Female						
0	24.6±1.1	25.8±1.5	28.9±1.6	29.1±1.6	4.8±1.2	
50	24.2±1.2	25.1±1.5	27.5±1.9	27.2±2.0	3.2±1.2	67
300	24.2±1.2	24.4±1.3*	27.8±2.1	28.5±2.7	4.6±2.1	96
1000	24.0±1.2	24.2±1.2*	27.5±1.4	28.1±2.0	4.2±2.1	88
7000	23.6±1.2	24.5±1.2*	27.2±1.9*	27.7±2.3	4.2±2.5	88

Data excerpted from pages 38-45 of the study report.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

No adverse, test substance-related effects on mean food consumption or food efficiency were observed in any male or female group. Mean overall food consumption (test days 0 to 91) in male and female 7000 ppm groups was 106% and 83% of control, respectively (statistically significant in females). Although the reduced overall food consumption in 7000 ppm female mice may have been test substance related, it was not considered adverse as it was not associated with statistically significant differences in food consumption over any weekly interval, and did not result in statistically significant decreases in mean final body weight, body or food efficiency. Statistically significantly lower mean overall food consumption was also observed in the 50 and 300 ppm female dose groups, but not in the 1000 ppm group, and only one interval (test days 7 to 14 in the 300 ppm group) demonstrated a statistically significant difference in weekly food consumption. Therefore, none of the significant differences in overall mean food consumption at these lower doses was considered test substance related. No statistically significant differences in food consumption were observed over any weekly interval in male mice.

Mean overall food efficiency (test days 0 to 91) in male and female 7000 ppm groups was 100% of control. Statistically significant reductions in mean food efficiency were observed over two weekly intervals in 50 ppm males, but were not considered test substance related as there was no dose-response observed at higher doses. Statistically significant differences in mean food efficiency were observed over several weekly intervals in all female dose groups except 50 ppm. These differences were not considered test substance-related, as they included both higher and lower food efficiency, and were not associated with a statistically significant difference in mean overall food efficiency.

D. OPHTHALMOLOGICAL EXAMINATIONS

No statistically significant or biologically significant changes in the incidences of ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Hematology

There were no test substance-related changes in hematology parameters in male or female mice. The platelet measurements were not reported for 3 groups of males and 1 group of males. The study report provided an explanation that acurate counts could not be quantitatively determined on most or all mice in all dose groups, due to platelet clumping (observed microscopically on peripheral blood smears), which caused the measured platelet counts to be lower than the actual values. However, platelet counts were generally found to have sufficient platelet numbers as evaluated microscopically (qualitative evaluation). Therefore, the test substance was considered to have had no effect on platelet counts.

2. Clinical chemistry

There were no treatment-related changes in plasma total protein, the only clinical chemistry parameter measured, in male or female mice. Minimally decreased plasma total protein in females fed 1000 ppm was considered to be unrelated to treatment because there was no dose-response.

F. PLASMA CONCENTRATION

IN-MLA84 was the most prevalent analyte present in male or female mice, followed by cyantraniliprole (Table 5). The metabolite, IN-MLA84, was greater than 2 orders of magnitude more prevalent than the parent cyantraniliprole. The level of this metabolite in the plasma of the low dose mice was greater than 100 mg/L. Assuming the plasma volume of a mouse to be 1.6 ml and the body weight of the test mouse to be 34 gm, the IN-MLA84 in the test rat was approximately 4.7 mg/kg bw which was more than half of the administered dose (7 mg/kg bw). At dose levels of 300 ppm or above, the plasma level of IN-MLA84 appeared to reach a plateau. Plasma values for this metabolite were similar between males and females at comparable diet concentrations. A detectable level of IN-MLA84 was also found in the control animals, and this was attributed to cross-contamination during blood collection and sample handling for analysis.

Table 5. Plasma concentrations of cyantraniliprole (DPX-HGW86) and four metabolites (ng/mL)

ppm	0	50	300	1,000	7,000
Males (ng/mL)					
DPX-HGW86	<LOQ	85±33	815±270	1,451±321	3,942±1,045
IN-J9Z38	<LOQ	<LOQ	64±18	118±17	278±45
IN-MYX98	<LOQ	26±4	179±29	308±75	839±197
IN-MLA84	241±76	111,500 ±11,385	394,100 ±27,898	402,800 ±33,320	411,000 ±34,943
IN-N7B69	<LOQ	<LOQ	68±14	121±29	262±51
Females (ng/mL)					
DPX-HGW86	<LOQ	140±108	1,002±230	2,634±1,073	8,980±9,857
IN-J9Z38	<LOQ	<LOQ	63±24	182±77	312±73
IN-MYX98	<LOQ	36±16	132±25	334±113	769±147
IN-MLA84	350±87	153,200 ±16,243	321,200 ±33,626	502,600 ±30,146	384,600 ±108,475
IN-N7B69	<LOQ	<LOQ	59±10	146±39	331±26

LOQ = 5 ng/mL Data excerpted from pages 15-16 of the report.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Mean liver weights were significantly increased in 7000 ppm males and ≥1000 ppm females. In the 7000 ppm males, mean absolute and relative (% body weight) liver weights were increased 24% and 26% as compared to controls (Table 6). In females, mean absolute liver weights were increased 7% and 15% in the 1000 ppm and 7000 ppm, respectively. Mean relative liver weights were increased 11% and 24% in the 1000 and 7000 ppm groups, respectively. The increased liver weights correlated with microscopic hepatocellular hypertrophy at the same concentrations. Both the liver weight increases and the hepatocellular hypertrophy were considered indicative of adaptive hepatic enzyme induction. All other individual and mean organ weight differences were interpreted as spurious or related to the non-statistically significant lower (8%) mean female final body weight at 7000 ppm. Statistically significant decreases in epidimydes and ovary weights were also found in 7000 ppm males and females, respectively (Table 6).

Table 6. Selective Organ weights					
ppm	**0**	**50**	**300**	**1000**	**7000**
Males					
Liver Absolute weight (g)	1.93±0.17	2.00±0.27	2.04±0.40	2.09±0.22	**2.39**±0.36*
Relative[a] to body weight (%)	4.8	4.8	5.1	5.2	**6.1***
Relative to brain weight (%)	374.7	397.8	397.7	409.2	**478.6***
Epididymides Absolute weight (g)	0.163±0.18	0.147±0.018	0.151±0.015	0.161±0.031	**0.141±0.016**+
Relative[a] to body weight (%)	0.41	0.35	0.38	0.41	0.36
Relative to brain weight (%)	31.6	29.3	29.5	31.4	28.1
Females					
Liver Absolute weight (g)	1.49±0.10	1.32±0.15	1.46±0.33	**1.59±0.18**	**1.7±0.25**
Relative[a] to body weight (%)	4.9	4.7	4.9	**5.4***	**6.1***
Relativeto brain weight (%)	301.5	268.0	293.9	**315.1**	**335.5**
Ovary Absolute weight (g)	0.039±0.010	0.032±0.007	0.032±0.010	0.036±0.011	**0.028±0.006**+
Relative[a] to body weight (%)	0.13	0.12	0.11	0.12	0.10
Relative to brain weight (%)	7.83	6.59	6.41	7.06	**5.39**+

[a] Organ to body weight ratio. Data excerpted from pages 67-72 of the study report.

* Significantly different from control by Dunnett/Tamhane/Dunnett test, p <0.05. +: Statically significant p< 0.05 by Dunn's test

Note: Bolded values were interpreted to be test-substance related increases, as compared to control values.

2. Gross pathology and histopathology

An increase in hepatocellular hypertrophy was observed in males at 7000 ppm and in females at ≥1000 ppm, and was characterized by an increase in the size of centrilobular hepatocytes (Table 7). The hypertrophy correlated with increased mean liver weight parameters in males and females at the same concentrations and was considered as adaptive effect. An increase in the incidence of focal necrosis of the liver was seen in7000 ppm females (4/10) (Table 8). One female each of 300 and 1000 ppm also had focal necrosis of the liver. Minimal inflammation of the liver was seen in the controls and treated groups. Focal cellular alteration was seen in one 7000 ppm male.

An increase in the incidence of adrenal cortical microvesiculation was observed in males at ≥50 ppm; the severity of this finding was graded minimal to mild. **The adrenal cortical microvesiculation was not present in females at any dose group**. In addition, in an adrenal mechanistic study in mice (MRID 48119985), increased incidence of microvesiculation was not found in 7000 ppm males, and cyantraniliprole treatment did not produce adrenal cytotoxicity or degeneration with electron microscopy. In addition, no adrenal functional deficit was found (i.e., urine corticosterone levels were unchanged with treatment). Furthermore, increased incidence

of adrenal cortical microvesiculation was not found in the mouse carcinogenicity study which tested up to 7000 ppm (MRID 48122578). When these findings were considered together, at this time, the toxicological significance of this finding is not clear; however, it appears to be treatment related in the 90-day study.

Table 7. Incidences of microscopic effects on the liver and the adrenal gland

ppm	0	50	300	1000	7000
Males					
Centrilobular hepatocellular hypertrophy	0/10	0/10	0/10	0/10	**2/10**
Adrenal microvesiculation (zona fasciculate)	0/10	**3/10**	**5/10**	**4/10**	**7/10**
Females					
Centrilobular hepatocellular hypertrophy	0/10	0/10	0/10	1/10	**9/10**
Adrenal microvesiculation (zona fasciculate)	0/10	0/10	0/10	0/10	0/10

Data excerpted from pages 94-145 of the report (Incidence and lesion grades of microscopic observations-non-neoplastic).

Table 8. Additional histopathology findings in the liver of treated mice

ppm	0	50	300	1000	7000
Males					
Liver: focal necrosis, minimal	0/10	0/10	0/10	1/10	1/10
Inflammation, minimal	5/10	4/10	3/10	7/10	5/10
Focus of cellular alteration, basophilic	0/10	0/10	0/10	0/10	1/10
Fibrosis	0/10	0/10	0/10	0/10	1/10
Females					
Liver: focal necrosis, minimal	0/10	0/10	1/10	1/10	4/10
Inflammation, minimal	5/10	4/10	3/10	7/10	5/10
Focus of cellular alteration, basophilic	0/10	0/10	1/10	0/10	0/10
Fibrosis	0/10	0/10	0/10	0/10	1/10

Data excerpted from pages 94 & 106 of the study report.

III. CONCLUSION

When Crl:CD1®(ICR) mice were fed cyantraniliprole at concentrations of 0, 50, 300, 1000, and 7000 ppm for 90 days, treatment-related changes in liver weights were seen in 1000 and 7000 ppm females and 7000 ppm males. Centrilobular hepatocellular hypertrophy was found in 7000 ppm males and females. Increases in liver weights and hypertrophy were considered as adaptive response due to increases in liver metabolizing activity and liver enzymes as shown in the 28-day toxicity study im mice (MRID 48119940). However, there was an increase in the incidence of focal necrosis of the liver in the 7000 ppm females (4/10) and males (1/10). Focus of cellular alteration was also seen in one 7000 ppm male. Statistically significant decreases in epididymides and overy weights were also found in 7000 ppm males and females, respectively. Histopathological changes were not seen in testes and ovary. The toxicological significant of this finding is not clear because in the carcinogenicity study both ovary and epididymides were not affected at similar concentration (7000 ppm). Therefore, the NOAEL was 1000 ppm (150/204 mg/kg bw/day for males/females); LOAEL was 7000 ppm (1092/1344 mg/kg bw/day for males/females) based on focal necrosis of the liver along with liver increases.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.2.1 Oral 90-day toxicity in the rat

IIA 5.3.2/01

Report: Carpenter, C. (2007); DPX-HGW86 technical: Subchronic toxicity 90-day feeding study in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-16993. July 23, 2007. MRID 48119945. Unpublished.

IIA 5.3.2/02

Report: Gannon, S.A. (2011a); DPX-HGW86 technical: Subchronic toxicity 90-day feeding study in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-16993, Supplement No.1, Revision No. 1. April 16, 2011. MRID 48119946. Unpublished.

Guidelines: OPPTS 870.3100 (1998)
OECD 408 (1998),
Commission Directive 2001/59/EC Part B.26 (2001),
MAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 90-day feeding study (MRID 48119945), cyantraniliprole (93.4%; HGW86-141) was administered to male and female Crl:CD®(SD) rats (10 rats/sex/concentration) at concentrations of 0, 100, 400, 3000, and 20000 ppm (males: 0, 6, 22, 168, or 1147 mg/kg bw/day; females: 0, 7, 27, 202, or 1346 mg/kg bw/day). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, hematology, clinical chemistry, urinalysis, ophthalmology, organ weights, gross and microscopic pathology. Five satellite groups of young adult male and female Crl:CD®(SD) rats (5/sex/group) were administered the same dietary concentrations of cyantraniliprole for 29 days (males) or 30 days (females); these groups of test animals were term "28-day sacrifice animals". Livers, thyroid glands, and blood were collected. Serum thyroid hormone concentrations and hepatic biochemical parameters (cytochrome P450 and UDP-glucuronyltransferase) were evaluated after approximately 28 or 90 days of diet administration of the test substance. In a supplemental study (MRID 48119946), blood samples were collected from the test animals of the main study on test days 63 (males) and 64 (females); they were analyzed and quantified for the concentration of the parent compound and metabolites (IN-JZ38, IN-MY98, IN-MLA84, and IN-N7B69) in plasma.

Under the conditions of this study cyantraniliprole did not produced test substance-related deaths, clinical signs, ophthalmological observations, changes on body weights or nutritional parameters, clinical pathology parameters (hematology, clinical chemistry, coagulation parameters, urinalysis), or gross pathology in treated male and female rats.

Increases in liver weight were observed in males of ≥3000 ppm at 28 days and of ≥400 ppm at 90 days. In females, liver weights were increased at ≥400 ppm at both 28 and 90 days. The increases in liver weight were accompanied by centrilobular hepatocellular hypertrophy in male (≥3000 ppm) and female (≥400 ppm) at 90-day and in female (≥3000 ppm) at 28-day. Similarly, total hepatic cytochrome P-450 content was increased in males at 28-day (400, 3000 and 20000 ppm) and 90-day (3000 and 20000 ppm) time points and in females at the 90-day time point (20000 ppm). The effects on the liver were considered as an adaptive response.

Thyroid follicular cell hypertrophy was observed in 90-day males fed 20000 ppm and in 90-day females fed 400, 3000 or 20000 ppm and was regarded as adverse. The thyroid hypertrophy correlated with alterations in thyroid hormone concentrations and induction of UDP-glucuronyltransferase activity and, in females, increased mean thyroid weights at these dietary levels (400, 3000, and 20000 ppm). Alterations in thyroid hormone concentrations were also observed in male rats at 400 and 3000 ppm, but were not considered adverse as there was no correlative histopathology or alterations in UDP-glucuronyltransferase activity. Mean relative and absolute thyroid weights were increased (variable statistical significance) in the 28-day sacrifice 20000 ppm female group. No adverse effects were observed on organ weights in male 28-day sacrifice rats or on gross or microscopic parameters in male or female 28-day sacrifice rats. A minimal to mild increase in adrenal cortical microvesiculation in male rats in the 90-day sacrifice 20000 ppm males was considered to be a non-adverse change within normal physiological limits.

The results of the supplemental study showed that the most abundant analyte present in the plasma of male and female rats was IN-MLA84 followed by parent cyantraniliprole and IN-J9Z38. Concentrations were higher in females than in males at all dietary concentrations, except for IN-J9Z38, and the plasma concentrations approached a plateau above a dietary concentration of 400 ppm.

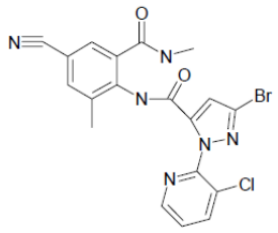
The no-observed adverse-effect level (NOAEL) for male 90-day rats was 3000 ppm (168 mg/kg bw/day). The LOAEL was 20000 ppm (1147 mg/kg bw/day) based on thyroid follicular cell hypertrophy, increased thyroid weights, and alterations in thyroid hormone homeostasis in male rats. **The NOAEL for female 90-day rats was 100 ppm (7 mg/kg bw/day). The LOAEL was 400 ppm (27 mg/kg bw/day) based on thyroid follicular cell hypertrophy, increased thyroid weights, and alterations in thyroid hormone homeostasis in female rats fed 400 ppm (27 mg/kg bw/day) and above**. It should be noted that the compound intakes in treated females were greater than those in males in every dietary concentrations by approximately 20%.

This study is classified as fully reliable (acceptable/guideline) and satisfies guideline requirements for a subchronic oral toxicity in rats [OPPTS 870.3100; OECD 408 (1998)].

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-
Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]phenyl]



Lot/Batch #: HGW86-141
Purity: 93.4%
Description: Off-white solid
CAS # 736994-63-1
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 7 days at room temperature, was distributed uniformly in the feed and was present in the feed at targeted concentrations. Batches were prepared every other week.

2. Vehicle and/or positive control: Untreated diet

3. Test animals
Species: Rat
Strain: Crl:CD®(SD)
Age at dosing: Approximately 61 days old
Weight at dosing: 270.6-321.0 g for males; 188.9-225.7 g for females
Source: Charles River Laboratories, Inc., Raleigh, NC
Acclimation period: 11 days
Diet: PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions
Temperature: 18–26°C
Humidity: 30–70%

Air changes: Not reported
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   06-June-2005 to 20-July-2007

2. Animal assignment and treatment

   Five groups of 10 animals/sex/concentration were administered concentrations of cyantraniliprole in feed daily for 92 (males) or 93 (females) days. Males and females received 0, 100, 400, 3000, and 20000 ppm. Five animals/sex/concentration were administered the same diet concentrations for 29 days (males) or 30 days (females) for mechanistic evaluations (Table 1). The 20000 ppm concentration is a limit dose level. The other concentrations were selected to establish a no-observed-adverse-effect level (NOAEL) and to assess a dose response for any observed effects.

   Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table1. Study Design and Compound Intakes

Conc. in diet (ppm)[a]	No./sex/ group[b]	Mean daily compound intakes[c] (mg/kg bw)	Mean daily compound intakes[c] mg/kg bw
0	15	0	0
100	15	6	7
400	15	22	27
3000	15	168	202
20000	15	1147	1346

[a]: Weight/weight concentration of the test compound (adjusted for 93.4% purity)
[b]: Five animals/sex/concentration were administered the same concentrations for 29 days (males) or 30 days (females) for mechanistic evaluations.
[c]: Data excerpted from pages 72-75 of the report. The values were rounded to whole numbers.

3. Diet preparation and analysis

   The test substance was added to the rodent diet and thoroughly mixed for 3 minutes. Control diets were mixed for the same period of time. All diets were prepared every other week and refrigerated until used. The stability, homogeneity, and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC near the beginning and end of study. The test substance was at target concentrations (92.8 to 100.2% of nominal), homogeneous (88.0 to 105.7% of nominal) throughout the feed and was stable (average percentage of active ingredient was 94.2% ± 1.4 and 92.2% ± 0.7) for up to 14 days at room temperature. Based on this information, it can be concluded that

the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics: Statistical methods employed in this study are listed in Table 2.

Table 2. Statistics used in data analyses.

Parameter	Preliminary test	Method of statistical analysis	
		If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Percent decrease in body weight gain Food consumption Food efficiency Clinical pathology[a] Organ weight[b] Cytochrome P450 Hormone levels	Levene's test for homogeneity and Shapiro-Wilk test for normality[c]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Survival Incidence of clinical observations Incidence of ophthalmology observations	None	Cochran-Armitage test for trend[d]	

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with that bilirubin data.

[b] Relative organ weights (percent of final body weight and percent of brain weight [90-day animals only for brain weight]) were calculated. Final body weights determined just prior to necropsy were used in the assessment of organ weight changes.

[c] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[d] If the incidence was not significant, but a significant lack of fit occurs, then Fisher's Exact test with a Bonferroni correction was used.

C. METHODS

1. Observations

   Animals were observed twice daily for mortality and morbidity, checked daily for acute signs of systemic toxicity, and examined weekly for detailed clinical signs of toxicity.

2. Body weights

   All animals were weighed once per week and prior to sacrifice.

3. Food consumption, food efficiency and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

   All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (hematology, clinical chemistry, coagulation, urinalysis)

Blood and urine samples were collected from all animals 38/39 and 92/93 days (males/females) after initiation of the study. Animals were fasted approximately 16 hours prior to sample collection. At sacrifice blood, bone marrow, and urine were collected. Hematology, clinical chemistry, coagulation, bone marrow smears, and urine analysis were all performed on the samples. The following parameters were examined.

Hematology

red blood cell count	red cell distribution width
hemoglobin	absolute reticulocyte count
hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular (cell) hemoglobin concentration	microscopic blood smear examination
prothrombin time	activated partial thromboplastin time

Clinical Chemistry

aspartate aminotransferase	glucose
alanine aminotransferase	total protein
sorbitol dehydrogenase	albumin
alkaline phosphatase	globulin
total bilirubin	calcium
urea nitrogen	inorganic phosphorus
creatinine	sodium
cholesterol	potassium
triglycerides	chloride
total bile acids	

Urinalysis

quality	ketone
color	bilirubin
clarity	blood
volume	urobilinogen
specific gravity	protein
pH	microscopic urine sediment examination
glucose	

6. Biochemistry/mechanistic parameters

Cyantraniliprole was evaluated for its ability to alter serum thyroid hormone concentrations, hepatic UDP-glucuronyltransferase activity, and total cytochrome P450 content. Serum hormone concentrations and hepatic microsomal parameters were evaluated after approximately 28 or 90 days of dietary administration of the test substance.

Blood was collected *via* the tail vein of male and female rats designated for hormonal analyses on test days 24 and 25 for the 28-day timepoint, and on test days 87 and 88 for the 90-day timepoint, respectively. Serum was prepared and stored between -65 and -85°C until analyzed for tri-iodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH). Serum hormone concentrations were measured by commercially-available radioimmunoassay (RIA) kits.

Livers were collected at necropsy from the male and female rats on test days 29 and 30 for the 28-day timepoint, and on test days 92 and 93 for the 90-day timepoint. A portion of the liver from each rat was homogenized (1 gram tissue/4 mL buffer) and hepatic microsomes were prepared using differential centrifugation. The resulting microsomal pellets were resuspended in the homogenization buffer, aliquot, and stored between -65 and -85°C until analyzed for UDP-glucuronyltransferase activity and total cytochrome P450 content. The protein content of the microsomes was determined before and after analysis by the Biorad method. The microsomal suspensions were diluted to a final protein concentration of approximately 1 mg/mL for analysis. Hepatic UDP-glucuronyltransferase activity was measured spectrophotometrically using ρ-nitrophenol as the substrate according to the methods of McClain and coworkers. Total cytochrome P450 content was measured spectrophotometrically according to the method of Omura and Sato. Final calculations were made using the post-assay protein concentrations.

7. Plasma concentration

Blood (non-fasted) was collected on test day 63 (males) and 64 (females), for determination of plasma concentration of test substance and selected metabolites. Samples were evaluated by liquid chromatography-mass spectroscopy (LC-MS).

8. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (20000 ppm) and control (0 ppm) and issues from an early decedent were processed to slides and evaluated microscopically. Gross observations, recorded at necropsy, were examined microscopically for all animals. Suspect target organs (liver, thyroid, and adrenals), as determined by examination of the control and high-dose rats, were processed to slides and examined microscopically for all rats.

Table 3. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Brain	X	X
Spleen	X	X
Heart	X	X
Liver	X	X
Kidneys	X	X
Adrenal glands	X	X
Thymus	X	X
Aorta		X
Testes	X	X
Epididymides	X	X
Prostate		X
Seminal vesicles		X
Ovaries (including oviducts)	X	X
Uterus (including cervix)	X	X
Mammary glands (females)		X
Stomach		X
Bone marrow		X
Pituitary		X
Trachea		X
Thyroid glands	X	X
Parathyroid glands		X
Nose		X
Skeletal muscle		X
Lungs		X
Larynx/pharynx		X
Spinal cord		X
Sciatic nerve		X
Femur/knee joint		X
Sternum		X
Skin		X
Eyes		X
Esophagus		X
Urinary bladder		X
Jejunem		X
Mesenteric lymph node		X
Mandibular lymph node		X
Peyer's patches		X
Duodenum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Gross observations		X

[a] All tissues from animals in the highest dose and control groups were evaluated. Suspect target organs (liver, thyroid, adrenals) were processed to slides and examined microscopically for all rats.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs were observed. The clinical signs noted did not exhibit a dose response and no statistically significant increase in incidence was observed.

2. Mortality

One male rat in the 400 ppm group was accidentally killed on test day 87. All remaining animals survived until the scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weight on test day 91 in male and female 20000 ppm groups was 94% and 96% of control, respectively (neither statistically significant) (Table 4). Mean overall (test days 0 to 91) body weight gain in male and female 20000 ppm groups was 90% and 86% of control, respectively; however, these reductions did show statistically significant difference from the control. Statistically significant decreases in mean body weight gains were observed in the interval days 35 to 42 in male rats fed 3000 or 20000 ppm and in the interval days 63 to 70 in male rats fed 20000 ppm. There were decreases in mean body weight gains on days 0 to 7 in female rats fed 3000 or 20000 ppm (Table 5). Although they indicated a statistically significant difference from the control, the reductions were not dose- related. No statistically significant difference in mean body weight gains were observed among groups over the interval days 0 to 28. The overall data indicated that cyantraniliprole did not produce adverse effects on the body weight and body weight gain.

Table 4. Mean body weights (gm)

ppm	0	100	400	3000	20000
Males					
Days 7	347±13	344±17	348±13	347±13	344±16
Days 28	449±29	447±32	456±21	443±18	442±30
Days 91	611±43	604±47	610±41	584±41	575±62
Females					
Days 7	226±14	223±13	224±12	221±10	222±8
Days 28	258±24	249±21	253±17	252±16	247±10
Days 91	303±46	290±35	297±25	298±27	291±19

Data excerpted from pages 56-59 of the report.

Table 5. Mean body weights gains (gm)

ppm	0	100	400	3000	20000
Males					
Days 0-7	48.1±6.8	47.7±8.6	51.5±4.6	51.4±5.7	49.5±8.6
Days 0-28	149.4±21.5	148.5±23.9	159.6±15.9	147.6±15.2	147.0±24.2
Days 0-91	310.6±33.5	306.3±40.3	311.6±41.5	287.1±41.4	279.7±44.8

Table 5. Mean body weights gains (gm)					
ppm	**0**	**100**	**400**	**3000**	**20000**
Females					
Days 0-7	19.6±7.2	18.8±6.9	15.4±6.2	10.8±5.3*	13.5±4.4*
Days 0-28	52.2±18.8	44.9±15.4	44.9±13.2	41.1±10.5	38.6±6.4
Days 0-91	95.5±41.2	86.4±27.9	88.0±20.3	85.2±22.3	81.9±18.1

*: Statistically significant ($p<0.05$) by Dunnett/Tamhane-Dunnette test
Data excerpted from pages 60-63 of the report.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

No test substance-related effects on mean food consumption or food efficiency were observed in any male or female group.

Mean overall food consumption (test days 0 to 91) in male and female 20000 ppm groups was 99% and 97% of control, respectively (neither statistically significant) (Table 6). Statistically significant decreases in mean food consumption were observed in the interval days 35 to 42 in male rats fed 3000 ppm or 20000 ppm. These differences were not considered test substance-related, as they did not exhibit a dose response and were not associated with a statistically significant difference in mean overall food consumption.

Mean overall food efficiency (test days 0 to 91) in male and female 20000 ppm groups was 91% and 93% of control, respectively (neither statistically significant) (Table 7). Statistically significant decreases in mean food efficiency were observed in the interval days 35 to 42, 63 to 70, and 28 to 91 in male rats fed 20000 ppm. Statistically significant decreases in mean food efficiency were observed in the interval days 0 to 7 in female rats fed 3000 or 20000 ppm, but the decreases did not show a dose-related response. A statistically significant increase in mean food efficiency was observed in the interval days 28 to 35 in female rats fed 20000 ppm. These changes in food efficiency were inconsistent and not dose-related. Therefore, the changes were not compound- related.

Table 6. Mean food consumption(gm/animal/day)					
ppm	**0**	**100**	**400**	**3000**	**20000**
Males					
Days 0-7	28.0±2.2	28.9±2.7	27.9±1.9	27.7±1.6	27.7±2.5
Days 0-28	27.4±1.8	27.8±2.5	27.4±1.7	27.3±1.4	27.5±2.4
Days 0-91	27.9±1.8	28.0±2.1	28.0±1.7	27.3±1.8	27.5±2.4
Females					
Days 0-7	19.2±1.6	19.3±2.5	18.1±1.5	18.4±3.3	19.0±2.2
Days 0-28	18.6±1.9	18.5±1.5	18.2±1.5	18.0±1.4	18.1±1.2
Days 0-91	18.4±2.0	18.0±1.4	18.0±1.3	18.2±1.2	17.8±0.8

*: Statistical significance ($p<0.05$). Data excerpted from pages 64-67 of the study report.

Table 7. Food efficiency (gm body weight gain/food consumed)					
ppm	**0**	**100**	**400**	**3000**	**20000**
Males					
Days 0-7	0.246±0.028	0.236±0.038	0.264±0.022	0.266±0.029	0.256±0.028
Days 0-28	0.195±0.024	0.190±0.020	0.208±0.019	0.193±0.018	0.190±0.022
Days 0-91	0.122±0.010	0.12±0.008	0.122±0.011	0.115±0.012	0.111±0.012
Females					
Days 0-7	0.143±0.042	0.139±0.015	0.119±0.042	0.084±0.040*	0.102±0.033*
Days 0-28	0.98±0.027	0.086±0.025	0.088±0.024	0.081±0.018	0.076±0.011*
Days 0-91	0.055±0.018	0.051±0.014	0.053±0.010	0.051±0.011	0.051±0.010

*: Statistical significance ($p<0.05$). Data excerpted from pages 68-71of the study report

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related ophthalmological findings were observed in any male or female group.

E. CLINICAL PATHOLOGY

1. Hematology

There were no adverse or statistically significant changes in hematologic parameters in male or female rats. The individual animal data showed that there were certain changes in red blood cell morphology observed microscopically on blood smears of both controls and treatment groups. The incidence included echinocytes (red blood cells with short surface projections), polychromasia, anisocytosis (unequal size RBC), microcytosis, and macrocytosis; these shape and size changes were seen in both controls and treated animals. The presence of these changes in various treatment levels showed no dose-related response (Tables 7a). However, at 3000 and 20000 ppm the degree of these changes appeared to be increased (i.e, changes at lower dose groups were graded as trace and few; at 3000 and 20,000ppm groups were reported to have moderate and many). These shape and size changes were observed in the absence of changes in other red cell parameters such as red cell mass, red blood cell count, hemoglobin, and hematocrit. The shape changes did not appear to be adverse but might be influenced by treatment at 3,000 and 20,000 ppm groups.

Table 7a. Incidence and grade/degree of echinocytes and acanthocytes (day 92 for males day 93 for females)					
ppm	**0**	**100**	**400**	**3000**	**20000 ppm**
Males (n=10)					
Echinocytes	3	6	5	7	8
trace	2	2	3	3	4
few	1	2	1	2	1
many					1
moderate		2	1	2	2

Table 7a. Incidence and grade/degree of echinocytes and acanthocytes (day 92 for males day 93 for females)					
ppm	**0**	**100**	**400**	**3000**	**20000 ppm**
Acanthocytes	8	9	9	9	10
trace	1				
few	1	2	5	2	2
many	2	1	2	3	2
moderate	4	6	2	4	6
Females (n=10)					
Echinocytes	8	9	10	9	10
trace	4	1	1		
few		5	3	1	1
many	3	2	5	6	8
moderate	1	1	1	2	1
Acanthocytes	7	9	8	6	6
trace	2	2	3	2	4
few	4	7	3	4	2
many					
moderate	1		2		

Data excerpted from the individual animal data on pages 320-322 (males) and 326-328 (females) of the study report.

2. Clinical chemistry

As shown in Table 8, there were some slight changes in clinical chemistry parameters relative to the controls, and some changes demonstrated statistical significant difference from the controls. However, none of these changes showed a clear dose-related response, and they were not considered to be adverse. The individual changes are discussed below.

Table 8. Selective clinical chemistry parameters which presented changes						
Ppm		**0**	**100**	**400**	**3,000**	**20,000**
Males						
Bilirubin (mg/dL)	Day 39	0.14±0.03	0.12±0.01	0.11±0.02*	0.09±0.02*	0.09±0.01*
	Day 93	0.14±0.02	0.12±0.02*	0.11±0.01*	0.11±0.02*	0.09±0.02*
Urea nitrogen (mg/dL)	Day 39	13±2	13±2	13±2	12±2	11±1*
	Day 93	13±2	12±1	13±2	12±1	12±2
Females						
Bilirubin (mg/dL)	Day 39	0.18±0.04	0.13±0.01*	0.09±0.02*	0.09±0.01*	0.09±0.01*
	Day 93	0.017±0.04	0.12±0.01	0.09±0.02[a]	0.09±0.01[a]	0.08±0.01[a]
Cholesterol (mg/dL)	Day 39	79±18	81±23	85±18	78±16	95±20
	Day 93	83±19	92±16	99±27	87±19	112±26*

Table 8. Selective clinical chemistry parameters which presented changes						
Ppm		**0**	**100**	**400**	**3,000**	**20,000**
Albumin (gm/dL)	Day 39	4.3±0.2	4.1±0.2	4.2±0.3	4.1±0.3	4.0±0.2*
	Day 93	4.3±0.5	4.3±0.1	4.3±0.4	4.1±0.4	4.1±0.2
Triglycerides (mg/dL)	Day 39	48±12	39±14	44±14	36±4	36±7
	Day 93	56±23	42±11	50±18	40±16	34±6 [a]
Globulin (gm/dL)	Day 39	3.3±0.3	3.4±0.2	3.5±0.3	3.5±0.2	3.6±0.2*
	Day 93	3.4±0.2	3.5±0.2	3.7±0.3*	3.6±0.3	3.7±0.2*

*: Statistically significant ($p<0.05$) by Dunnett/Tamhane-Dunnett test.
[a]: Statistically significant ($p<0.05$) by Dunn's test.
Data excerpted from pages 89-94 of the report.

- Cholesterol was minimally increased at test day 93 in females fed 20000 ppm (135% of control). Similar increase was not seen in males. In addition, cholesterol concentrations of several other females in other groups (including control) were similar to those of the high dose group (with the exception of one high value in a 20000 ppm female). Therefore, this change was considered to be likely related to treatment, but non-adverse because of the minimal degree of change.

- Triglycerides were moderately decreased at test day 93 in females fed 20000 ppm (61% of control mean). Triglyceride concentrations were also decreased (not statistically significant) in females fed 3000 ppm (test days 39 and 93) and 20000 ppm at test day 39 (means were 71 to 75% of respective control). These changes were possibly related to treatment. However, with the exception of a few values, the range of individual values was similar across the various dose groups despite the 200-fold difference in dietary concentration. These changes were considered to be non-adverse because individual values of females fed ≥3000 ppm were generally within the range of those observed in control and low dose animals.

- Bilirubin was mildly to moderately decreased at test days 38/39 and 92/93 in males and females in all groups fed the test substance (≥100 ppm). Bilirubin concentrations of rats fed ≥400 ppm were between 47% and 79% of control group means. Rats fed the test substance also had increased liver weights and/or centrilobular hypertrophy (males and females fed ≥400 ppm), suggesting enzyme induction. Changes in bilirubin were considered to be secondary to increased metabolism of bilirubin caused by enzyme induction. Therefore, decreases in serum bilirubin were considered to be treatment-related and secondary to enzyme induction but not adverse, because mildly to moderately decreased bilirubin has no adverse consequences.

- Urea nitrogen was transiently and minimally decreased on test day 38 in males fed 20000 ppm. This change was unlikely to be related to treatment, as no such decrease was seen in males fed 20000 ppm at the termination of the study (day 92). Regardless, minimally decreased urea nitrogen has no toxicological significance; therefore, this change was considered to be non-adverse.

- Albumin was statistically decreased at test day 39 in females fed 20000 ppm. This change was not present at the end of dosing in a previous 28-day feeding study at this same concentration (WR 15230, SC 880). In addition, the change did not progress with further dosing (no substantial difference between values at test days 39 and 93). There were no changes in albumin for males, and there were no correlative changes in histology. Therefore, these changes were considered to be unrelated to treatment.

- Globulin was statistically increased at test days 39 and 93 in females fed 20000 ppm. This change was not present at the end of dosing in a previous 28-day feeding study at this same concentration. In addition, the change did not progress with further dosing (no substantial difference between values at test days 39 and 93). There were no changes in globulin concentrations for males, and there were no correlative changes in histology. Therefore, these changes were considered to be unrelated to treatment.

3. Coagulation

There were no adverse or treatment-related changes in coagulation parameters in treated male or female rats.

4. Urinalysis

In 20,000 ppm males, urine volume was minimally and transiently increased and urine concentration (osmolality) was mildly decreased at test day 38, though no such finding was seen in males at study termination (day 92). Additionally, this change was not present at the end of dosing in a previous 28-day feeding study at this same concentration. There were no changes in other clinical pathology or histology parameters associated with this change. These changes were unlikely to be related to treatment.

F. BIOCHEMISTRY/MECHANISTIC PARAMETERS

28-Day sacrifice

Following 28-day of treatment with cyantraniliprole, thyroid hormone homeostasis was altered with related changes in liver enzyme activity and P450 content in both male and female rats as shown in Table 9.

Table 9. Thyroid and biochemistry parameters (28-day sacrifice)

Parameter	0	100	400	3000	20000
Males					
T_4 (µg/dL)	3.7±0.9	3.1±0.7	2.3± 0.5* (↓38 %)	2.1±0.3* (↓43%)	2.1±0.7* (↓43%)
T_3 (ng/dL)	70.4±15.0	68.9±16.4	60.6±15.0 (↓14%)	61.0 ±23.8 (↓13%)	55.7±18.4 (↓21%)
TSH (ng/mL)	6.8±1.5	6.5±1.4	6.7±2.2	8.2±2.9 (↑21%)	8.3±5.1 (↑22%)
Total P450 (nmol/mg protein)	0.68± 0.16	0.68±0.12	0.80±0.20 (↑18%)	0.81±0.08 (↑19%)	0.93±0.15* (↑37%)
UDP-GT (nmol/min-mg)	31.2± 3.3	40.7±7.2	45.4±12.7* (↑46%)	45.7± 3.9* (↑46%)	55.4±8.1* (↑78%)
Females					
T_4 (µg/dL)	3.0±0.6	2.6±0.8	2.1± 0.7* (↓30%)	1.4± 0.5* (↓53%)	1.3± 0.5*(↓57%)
T_3 (ng/dL)	81.3±11.0	79.2±11.8	62.3± 6.2* (↓23%)	68.1±15.9 (↓16%)	63.3±17.4* (↓22%)
TSH (ng/mL)	5.9±1.6	5.7± 0.8	5.9± 1.4	6.0± 1.2	5.9± 1.4
Total P450 (nmol/mg protein)	0.48±0.06	0.47± 0.04	0.46± 0.08	0.50±0.06	0.56± 0.06 (↑17%)
UDP-GT (nmol/min-mg)	31.6± 2.6	40.1± 4.5	61.2± 20.0* (↑94%)	59.9±7.7* (↑90%)	66.4± 12.2* (↑110%)

* Significantly different from control, $p < 0.05$. Data excerpted from pages 204-207 of the report.

Males

In male rats, serum T_4 concentrations were statistically significantly decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (38, 43, and 43% decrease, respectively) (Table 9). Although not statistically significant, serum T_3 concentrations were decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (14, 13, and 21%, respectively). As expected, serum TSH concentrations were increased at dietary concentrations of 3000 and 20000 ppm cyantraniliprole (21 and 22% of control, respectively), but this increase did not showed a statistical significance. The effects on serum T_3 and TSH concentrations were considered test substance-related effects due to the corroborative changes in serum T_4 concentrations and hepatic UDP-glucuronyltransferase activity.

The alterations in thyroid hormones in male rats were accompanied by increased total hepatic cytochrome P450 content and UDP-glucuronyltransferase activity. Total hepatic cytochrome P450 content was increased at dietary concentrations of 400, 3000, and 20000 ppm (18, 19, and 37% above the control, respectively) and was statistically significantly increased at 20000 ppm. Hepatic UDP-glucuronyltransferase activity was numerically increased at all dietary concentrations and was statistically significantly increased at 400, 3000, and 20000 ppm (46, 46, and 78% greater than the control, respectively). In addition, the biochemical and hormonal effects in male rats at the 28-

day time point correlated with increased liver weight at dietary concentrations of 3000 and 20000 ppm.

Females

In female rats with the 28-day treatment, the effects were more pronounced than those observed in the male rats. Serum T_4 concentrations were statistically significantly decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (30, 53, and 57% decrease, respectively) (Table 9). Consistent with the effects on T_4, serum T_3 concentrations were numerically decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (23, 16, and 22% decrease, respectively) and showed statistical significance at 400 and 20000 ppm. Unlike male rats, serum TSH concentrations were not affected in female rats at the 28-day.

The alterations in thyroid hormones in female rats were also accompanied by increased hepatic UDP-glucuronyltransferase activity. Hepatic UDP-glucuronyltransferase activity was increased at all dietary concentrations of cyantraniliprole, and was statistically significantly increased at 400, 3000, and 20000 ppm cyantraniliprole (94, 90, and 110% greater than the control, respectively). Total hepatic cytochrome P450 content was not affected. The biochemical and hormonal effects in female rats at the 28-day timepoint correlated with increased relative liver weight and increased incidence of hepatocellular hypertrophy at dietary concentrations of ≥3000 ppm.

90-Day terminal sacrifice

Overall, similar patterns of changes in the thyroid hormones, TSH, liver enzyme P450, and liver UDP-glucuronyltransferase activity at the 90-day evaluation as those seen in the 28 day duration (Table 10).

Table 10. Thyroid and biochemistry parameters (90-day sacrifice)

ppm	0	100	400	3000	20000
Males					
T_4 (µg/dL)	4.1±0.7	3.7± 0.5	3.1± 0.8* (↓24%)	2.8± 0.5* (↓ 32%)	2.9± 0.6** (↓30 %)
T_3 (ng/dL)	67.4±5.3	64.5±9.8	55.7±11.6* (↓17%)	53.6± 9.4* (↓20%)	52.4±0.5** (↓22 %)
TSH (ng/mL)	7.1±1.0	8.2±2.3	7.9±2.1 (↑11%)	10.1± 4.1 (↑41%)	11.0±2.0* (↑55%)
Total P450 (nmol/mg protein)	0.86±0.09	0.95±0.27	0.79±0.15 (↑8%)	1.15±0.17* (↑34%)	1.10± 0.08 (↑28%)
UDP-GT (nmol/min-mg)	51.7±14.5	48.0±4.5	44.9±6.6	66.7± 8.8 (↑30%)	75.5± 8.4* (↑46%)
Females					
T_4 (µg/dL)	2.4± 0.6	2.0±0.7	1.4±0.6 (↓58%)	0.8±0.7* (↓67%)	0.5±0.5* (↓79%)
T_3 (ng/dL)	92.1± 7.9	91.1±7.9	86.0±16.3 (↓7%)	63.3±13.3* (↓31%)	65.0±17.1* (↓29%)

Table 10. Thyroid and biochemistry parameters (90-day sacrifice)

ppm	0	100	400	3000	20000
TSH (ng/mL)	6.5±0.7	6.9±1.1	6.8±1.0 (↑5%)	6.7±1.1 (↑3%)	7.4±1.8 (↑14%)
Total P450 (nmol/mg protein)	0.55± 0.19	0.60±0.10	0.67±0.11 (↑22%)	0.70±0.14 (↑27%)	0.87±0.13* (↑58%)
UDP-GT (nmol/min-mg)	32.0±5.8	38.4± 9.1	51.2±8.0* (↑64%)	51.8±10.7* (↑62%)	67.7±13.1* (↑112%)

* Significantly different from control, p <0.05. Data excerpted from pages 208-211 of the report.

Males

Serum T_4 concentrations were statistically significantly decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (24, 32, and 30% decrease relative to the control, respectively). Similarly, serum T_3 concentrations were statistically significantly decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (17%, 20%, and 22% decrease, respectively). Serum TSH concentrations were numerically increased at dietary concentrations of 3000 and 20000 ppm cyantraniliprole (41, and 55% greater than the control, respectively), and were statistically significantly increased at 20000 ppm. The alterations in thyroid hormones were accompanied by increased total hepatic cytochrome P450 content and UDP-glucuronyltransferase activity at the 90-day timepoint.

Females

Overall, the effects observed in female rats at the 90-day timepoint were similar to the effects observed at the 28-day timepoint. Serum T_4 concentrations were numerically decreased at dietary concentrations of 400, 3000, and 20000 ppm cyantraniliprole (48, 673, and 79% decrease relative to the control, respectively), and were statistically significantly decreased at 3000 and 20000 ppm. Serum T3 concentrations were statistically significantly decreased at dietary concentrations of 3000 and 20000 ppm cyantraniliprole (31 and 29% lower than that of the control, respectively). Serum TSH concentrations were comparable between treated and the control groups. The alterations in thyroid hormones in female rats were accompanied by increased total hepatic cytochrome P450 content and UDP-glucuronyltransferase activity at the 90-day time point.

F. PLASMA CONCENTRATION

Plasma samples were analyzed simultaneously for parent cyantraniliprole and four metabolites (IN-J9Z38, IN-MYX98, IN-MLA84, and IN-N7B69). The concentration of each analyte was higher in females than in males at all dietary levels, except for IN-J9Z38 at the 20,000 ppm dietary level where male and female concentrations were approximately equal (Table11). The most abundant analyte present in plasma samples from both male and female rats at all dose concentrations was IN-MLA84. In males, the plasma concentration of IN-MLA84 was 145,890 ng/mL in the highest dose group (20000 ppm). The concentration of IN-MLA84 at this same dietary level in female rat plasma (259,500 ng/mL) was nearly

twice that of the males. The next most abundant analyte was parent cyantraniliprole. The concentrations of cyantraniliprole in plasma from male and female rats at the highest dietary level were 4634 ng/mL and 5624 ng/mL, approximately 30 and 46 times less than the concentration of IN-MLA84, respectively. In all cases the plasma concentration appears to approach a plateau above a dietary level of 400 ppm.

Table 11. Plasma concentrations of cyantraniliprole (DPX-HGW86) and four metabolites

ppm	0	100	400	3000	20000
Males (ng/mL)					
DPX-HGW86	14±4	357±64	1,729±754	3,402±552	4,634±761
IN-J9Z38	<LOQ	173±77	598±195[a]	1,298±590	1,464±304
IN-MYX98	<LOQ	29±15	110±26	207±70	455±102
IN-MLA84	<LOQ	16,303±5,758	67,500±13,216	91,605±21,906	145,890±30,076
IN-N7B69	<LOQ	16±4	21±9	35±20	50±25
Females (ng/mL)					
DPX-HGW86	<LOQ	1,592±399	4,245±1,232	6,010±1,717	5,624±1,679
IN-J9Z38	<LOQ	710±212	1,822±409	1,482±408	1,311±336
IN-MYX98	<LOQ	108±16	328±83	573±159	716±216
IN-MLA84	32±26[b]	29,150±6,457	175,300±4,0795	256,800±44,694	259,500±54,056
IN-N7B69	<LOQ	<LOQ	70±17	137±33	164±35

Limit of quantification (LOQ) = 5 ng/mL Data excerpted from page 15-19 of the report (MRID 48119946).
[a]: One outlier was not included in the calculation.
[b]: This value was due to cross-contamination during blood collection and sample handling.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

28-Day interim sacrifice (Table 12)

Liver

In males, mean absolute liver weights were increased 12% and 19% in the 3000 and 20000 ppm groups, respectively, as compared to the control values. Mean relative (% body weight) liver weights were also increased (12% and 19%, respectively). All, except for the increase in mean absolute liver weight in the 3000 ppm males, were statistically significant.

In females, mean absolute liver weights were increased 12%, 23%, and 27% in the 400, 3000, and 20000 ppm groups, respectively, as compared to the control values. Mean relative liver weights were also increased. All, except for the increases in the 400 ppm females, were statistically significant.

The increased liver weights correlated with microscopic hepatocellular hypertrophy only in females at ≥3000 ppm where the increase in mean absolute and relative liver

weight was greatest (≥23%). Since there were no adverse gross or microscopic findings that correlated with the increased liver weights, the liver weight effect, in both sexes, was considered an adaptive response due to enzyme induction.

Thyroid

In females, mean absolute thyroid weight was increased 91% in the 20000 ppm group as compared to the control value. Mean relative (% body weight) thyroid weight was increased 80%. Only the mean relative thyroid weight increase was statistically significant. In males, thyroid weight parameters in exposed rats were comparable those of the controls.

The increase in mean relative thyroid weight in the 20000 ppm female rats was considered potentially test substance related since it was both statistically significant and correlated with both the thyroid weight increase and microscopic effect (follicular cell hypertrophy) observed in the 90-day evaluation, described below.

Table 12 . Organ weights (28-day sacrifice)

ppm	0	100	400	3,000	20,000
Males					
Liver					
Absolute liver weight (g)	13.1±0.5	12.5±1.0	13.5±1.0	**14.7**±1.4 **(↑12%)**	**15.6**±1.1[b] **(↑19%)**
Relative[a] liver weight	3.2	3.0	3.2	**3.5**[b]	**3.8**[b]
Females					
Liver					
Absolute liver weight (g)	7.4±0.9	7.7±0.3	**8.3±1.5(↑12%)**	**9.2±0.7**[b]**(↑23)**	**9.4**±0.4[b]**(↑27)**
Relative[a] liver weight	3.1	3.2	**3.6**	**4.0**[c]	**4.0**[c]
Thyroid					
Absolute thyroid weight (g)	0.011±0.005	0.017±0.004	0.015±0.007	0.017±0.006	**0.021± 0.005 (↑91%)**
Relative[a] thyroid	0.005	0.007	0.006	0.008	**0.009**[c]**(↑80%)**

[a] Relative weight is defined as the organ to body weight ratio expressed as % body weight

[b] Significantly different from control by the Dunnett/Tamhane-Dunnett criteria, p <0.05.

[c] Significantly different from control by the Dunn's criteria, p <0.05.

Note: Bolded values were interpreted to be test substance related increases, as compared to control values
Data excerpted from pages 97-98 of the report.

90-Day terminal sacrifice

Liver

In males, mean absolute liver weights were increased only at 20000 ppm (13%), as compared to control values (Table 13). Mean relative (% body weight) liver weights were also increased in the 400, 3000, and 20000 ppm groups. All increases were statistically significant. In females, mean absolute liver weights were increased by

approximately 10%, 23%, and 34% in the 400, 3000, and 20000 ppm groups, respectively, as compared to the control weights. Mean relative liver weights were increased as well in these treated groups. All increases were statistically significant except for the mean absolute liver weight increase at 400 ppm.

The increased liver weights correlated with microscopic hepatocellular hypertrophy in all but the 400 ppm males. As in the 28-day examination period, the 90-day liver weight effect was considered as an adaptive response.

Thyroid

Thyroid weights were slightly increased in females following 90 days of dietary exposure to ≥400 ppm of the test substance. Mean absolute thyroid weights were increased 17%, 8%, and 17% in the 400, 3000, and 20000 ppm groups, respectively, as compared to control values. None of the increases were statistically significant. In males, absolute thyroid weights were comparable to that of the controls.

The slight increase in mean absolute thyroid weights in female rats at 400 ppm or greater was considered test substance related since it correlated with the microscopic thyroid follicular cell hypertrophy observed at the same doses. In addition, it correlated with the increased thyroid weights observed in the 28-day female rats fed 20000 ppm.

Table13. Selective Organ weights (90-day sacrifice)

ppm	0	100	400	3000	20000
Males					
Liver					
Absolute weight (g)	16.0±1.4	16.1±1.8	17.3±1.3	16.5±1.5	**18.0**±2.0[b]**(↑13%)**
Relative[a] weight (%)	2.7	2.8	**3.0**[b]	**2.9**[b]	**3.3**[b]
Kidney					
Absolute weight (g)	4.3±0.3	4.2± 0.4(97)	4.5± 1.0	3.9±0.3[c]	4.4±0.5
Relative[a] weight (%)	0.74	0.72	0.77	0.69	0.80
Thyroid					
Absolute weight (g)	0.032±0.005	0.028±0.006	0.033±0.007	0.034±0.007	0.034±0.008
Relative[a] weight (%)	0.005	0.005	0.006	0.006	0.006
Females					
Liver					
Absolute weight (g)	8.4±1.1	8.4±0.8	**9.2±1.1(↑10%)**	**10.3**±1.1[b]**(↑23%)**	**11.2**±0.9[b]**(↑33%)**
Relative[a] weight	2.9	3.0	**3.2**	**3.6**[b]	**4.1**[b]
Thyroid					
Absolute weight (g)	0.024±0.007	0.024±0.007	**0.028±0.004 (↑17%)**	**0.026±0.003 (↑8%)**	**0.028±0.005 (↑17%)**
Relative[a] weight (%)	0.009	0.009	0.010	0.009	**0.010**
Kidney					
Absolute weight (g)	2.21±0.02	2.09± 0.17	2.33±0.21	2.24±0.218	2.37±0.20
Relative[a] weight (%)	0.76	0.76	0.82	0.79	**0.86**[b]

[a] Relative weight is defined as the % of the body weight. [b] Statistically significant by the Dunnett/Tamhane-Dunnett criteria, $p<0.05$

[c] Significantly significant by the Dunn's criteria, $p < 0.05$. Data excerpted from pages 99 -104 of the report.
Note: Bolded values were interpreted to be test-substance related increases, as compared to control values.

Kidney

The mean relative (% of body weight) kidney weight was increased (13%) in the 20000 ppm females, as compared to the control value. The increase was statistically significant.

2. Gross pathology and histopathology

There were no test substance-related gross observations in rats from either the 28-day sacrifice or the 90-day sacrifice.

28-Day interim sacrifice (Table 14)

At the 28-day interim sacrifice, test substance-related microscopic findings were observed only in the liver (centrilobular hepatocellular hypertrophy) of 3000 and 20000 ppm females. The incidence was dose related and all were graded as minimal. The hepatocellular hypertrophy was characterized by an increase in the size of centrilobular hepatocytes. The hypertrophy correlated with increased mean liver weights at these dose levels.

Table 14. Incidences of microscopic effects (28-day sacrifice)

Ppm	0	100	400	3000	20000
Males					
Liver Hypertrophy, hepatocellular centrilobular	0/5	0/5	0/5	0/5	0/5
Females					
Liver Hypertrophy, hepatocellular centrilobular	0/5	0/5	0/5	**3/5**	**5/5**

Note: Bolded values were interpreted to be test-substance related increases, as compared to control values. Data excerpted from pages 146-147 of the report.

90-Day terminal sacrifice (Table 15)

Liver

At the 90-day sacrifice, centrilobular hepatocellular hypertrophy was observed in both males and females, and the incidence and severity were dose related. In males, hypertrophy was present in 0/10, 0/10, 0/10, 4/10, and 7/10 rats fed 0, 100, 400, 3000, and 20000 ppm, respectively, and all were graded as minimal (grade 1 of 4). In females, hepatocellular hypertrophy was present in 0/10, 0/10, 2/10, 6/10, and 9/10 rats

fed 0, 100, 400, 3000, and 20000 ppm, respectively. All were graded as minimal, except for one (1/6) 3000 ppm female and five (5/9) 20000 ppm females, which were graded mild (grade 2 of 4).

The hepatocellular hypertrophy was characterized by an increase in the size of centrilobular hepatocytes. The hypertrophy correlated with increased mean liver weight parameters in males and females fed ≥400 ppm of the test substance.

Thyroid

In the 90-day sacrifice, thyroid follicular cell hypertrophy was observed in both males and females and the incidence was dose related. In males, hypertrophy was present in 0/10, 0/10, 0/10, 0/10, and 5/10 rats fed 0, 100, 400, 3000, and 20000 ppm, respectively, and all were graded as minimal. In females, hypertrophy was present in 1/10, 0/10, 3/10, 4/10, and 6/10 rats fed 0, 100, 400, 3000, and 20000 ppm, respectively. All were graded as minimal.

The thyroid cell hypertrophy was characterized by an increase in the size of the follicular lining cell and a change in shape from flat or cuboidal cell to columnar. The microscopic hypertrophy correlated with increased mean thyroid weights in females (≥400 ppm).

Adrenal glands

A minimal to mild increase in microvesiculation of the zona fasciculata cells of the adrenal cortex was observed in 1/10, and 6/10 males of 3000 and 20000 ppm, respectively. The increase was graded as minimal (grade 1 of 4) in all but 2 high-dose males, which were graded as mild (grade 2 of 4). The increased microvesiculation was considered test substance related only at 20000 ppm.

In females, adrenal cortical microvesiculation was observed in 3000, and 20000 ppm at the incidence of 2/10 at both concentrations, but a dose-related-response was not present. Again, the effect was graded as minimal in all four rats. The incidence and severity of microvesiculation was within normal limits and was not considered to be test substance related.

The minimal to mild increase in adrenal cortical microvesiculation in males was within normal physiological limits and was considered to be a non-adverse change.

Table 15. Incidences of microscopic effects (90-day sacrifice)

ppm	0	100	400	3000	20000
Males					
Liver hypertrophy, hepatocellular centrilobular	0/10	0/10	0/10	**4/10**	**7/10**
Thyroid follicular cell Hypertrophy	0/10	0/10	0/10	0/10	**5/10**
Adrenal cortical microvesiculation (z.f.[b])	0/10	0/10	0/10	1/10	**6/10**

Table 15. Incidences of microscopic effects (90-day sacrifice)

ppm	0	100	400	3000	20000
Females					
Liver hypertrophy, hepatocellular centrilobular	0/10	0/10	**2/10**	**6/10**	**9/10**
Thyroid follicular cell hypertrophy,	1/10	0/10	**3/10**	**4/10**	**6/10**
Adrenal cortical microvesiculation (z.f.[b])	0/10	0/10	0/10	2/10	2/10

[b] z.f. = zona fasciculate. Data excerpted from pages 148-171 of the report.

Note: Bolded values were interpreted to be test substance related increases, as compared to control values.

III. CONCLUSION

Under the conditions of this study, cyantraniliprole produced no adverse or treatment-related effects on survival, clinical signs of toxicity, ophthalmological observations, changes on body weights, food consumption, food efficiency, clinical pathology parameters (hematology, clinical chemistry, coagulation parameters, urinalysis), or gross pathology in treated male and female rats. However, it caused adaptive response in the liver as demonstrated by increased liver weights, hepatic cytochrome P450 and hepatic UDP-glucuronyltransferase activity accompanied by increase incidence of liver hypertrophy (described as increase in the size of centrilobular hepatocytes). It produced adverse effects in the thyroid gland as indicated by deceases in the thyroid hormones (T_3 and T_4), increases in TSH and thyroid weight, and increased incidence of thyroid follicular cell hypertrophy.

The no-observed adverse-effect level (NOAEL) for male 90-day rats was 3000 ppm (168 mg/kg bw/day). The LOAEL was 20000 ppm (1147 mg/kg bw/day) based on thyroid follicular cell hypertrophy and alterations in thyroid hormone homeostasis in males. The NOAEL for female 90-day rats was 100 ppm (6.9 mg/kg bw/day). The LOAEL was 400 ppm (27 mg/kg bw/day) based on thyroid follicular cell hypertrophy, increased thyroid weights, and alterations in thyroid hormone homeostasis in female rats. It should be noted that the compound intakes in treated females were greater than those in males in every dietary concentrations by approximately 20%.

This study is classified as fully reliable (acceptable/guideline) and satisfies guideline requirements for a subchronic oral toxicity in rats [OPPTS 870.3100; OECD 408 (1998)].

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.3 Oral 90-day toxicity (dog)

IIA 5.3.3/01

Report: Luckette, E.M. (2007); DPX-HGW86 technical: 90-day dietary toxicity study in dogs MPI Research, Mattawan, Michigan, USA. Laboratory Report No**.:** 125-055. DuPont -16994, Revision No. 1. August 29, 2007. MRID 48119948. Unpublished.

IIA 5.3.3/02

Report: Gannon, S.A. (2009); DPX-HGW86 Technical: 90-day dietary toxicity study in dogs. MPI Research, Mattawan, Michigan, USA. Laboratory Report No.: 125-055. DuPont-16994, Supplement No.1, Revision No. 1. November 26, 2007. MRID 48119947. Unpublished. (This supplemental report provides information on analytical results for cyantraniliprole and its metabolites in the plasma samples collected from the 90-day toxicity study in dogs)

Guidelines: OPPTS 870.3150,
OECD 409,
MAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 90-day feeding study (MRID 48119948), cyantraniliprole (93.4%; HGW86-141) was administered in the diet to male and female beagle dogs (4/sex/concentration) at concentrations of 0, 30, 100, 1000, and 10000 ppm (males: 0, 1, 3, 32, and 281 mg/kg bw/day; females: 0, 1, 3.5, 34, and 294 mg/kg bw/day). Parameters evaluated included mortality and morbidity, body weight, body weight gain, food consumption, food efficiency, compound consumption, clinical signs, neurobehavioral signs, hematology, urinalysis, clinical chemistry, ophthalmology, organ weights, and gross and microscopic pathology. Blood samples were also collected on test day 57 (unfasted) and analyzed for concentrations of parent compound and metabolites (IN-J9Z38, IN-MY98, IN-MLA84, & IN-N&B69) (Supplemental report, MRID 48119947).

Treatment-related reductions in body weight, body weight gain, food consumption, and food efficiency were observed in male and female dogs at 10000 ppm; males were more severely affected than females. Adverse, test article-related changes in clinical chemistry were observed in ≥1000 ppm male and female dogs (decreased total protein and albumin concentrations, increased alkaline phosphatase activity) and 10000 ppm female dogs (increased alanine aminotransferase activity, decreased cholesterol). Liver weight was statistically increased in males and females at 1000 ppm and above. No adverse test article-related effects were observed in hematology, coagulation, or urinalysis parameters in any treatment group, and no adverse test

article-related effects on any clinical pathology parameter were observed at ≤100 ppm. Minimal bile duct hyperplasia was observed in male and female dogs at 10000 ppm and was considered test article related and adverse. At 10000 ppm, 2 males; 1 female were found to have arteritis (coronary and other arteries) and secondary effects in the myocardium.

The most abundant analyte in plasma was parent cyantraniliprole, which was present at comparable concentrations in both males and females at the same dietary concentrations. The concentration of other metabolites examined (IN-J9Z38, IN-MYX98, IN-MLA84, and IN-N7B69) was essentially negligible relative to that of the parent compound.

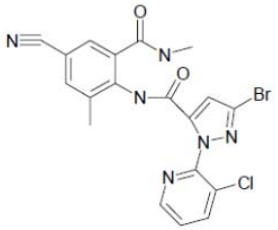
The no-observed-adverse-effect level (**NOAEL**) for cyantraniliprole in male and female dogs was 100 ppm (3 mg/kg bw/day for males and females). The **LOAEL** was 1000 ppm (32 mg/kg bw/day in males and 34 mg/kg bw/day in females) based on a collection of treatment-related effects indicative of hepatotoxicity. The effects included decreases in total protein, albumin, and cholesterol in males and females; increases in alkaline phosphatase in males and females, and increases in alanine aminotransferase in females, and increase liver weights in males and females.

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.3150) for a 90-oral toxicity study in dogs.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrazole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-141
Purity:	93.4%
Description:	Light white, solid
CAS #	736994-63-1

Stability of test compound:	Analysis confirmed that the test substance was considered to be at the targeted concentrations, homogeneously distributed in the diets, and stable when stored up to 14 days at room temperature or refrigerated.
2. Vehicle and/or positive control:	Untreated diet
3. Test animals:	
Species:	Dog
Strain:	Beagle
Age at dosing:	Approximately 6–7 months old
Weight at dosing:	6.40–8.80 kg for males; 5.05–6.94 kg for females
Source:	Covance Research Products, Inc., Kalamazoo, MI
Acclimation period:	2 weeks
Diet:	PMI® Nutrition International, LLC Certified Canine LabDiet® (#5007), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed two per cage (single sex) in double-sized stainless steel mobile cages in an environmentally controlled room.
4. Environmental conditions:	
Temperature:	64-84°F
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   27-September-2005 to 28-December-2005

2. Animal assignment and treatment

   Four groups of 4 animals/sex/concentration were administered concentrations of cyantraniliprole in feed daily for 90 days (Table 1). Males and females received 30, 100, 1000, and 10000 ppm. For females, animals considered suitable for study were weighed prior to treatment and randomized into treatment groups using a standard, by weight, block randomization procedure. For males, the animals were randomized into treatment groups based on testes volume. A negative control group received untreated diet.

Table 1. Study design and compound intake				
Group no.	**No./sex/ group**	**Conc. in diet (ppm)**	**Mean daily intakes[a] mg/kg bw**	
			Males	Females
1(control)	4	0	0	0
2	4	30	1	1
3	4	100	3	3.5
4	4	1000	32	34
5	4	10000	281	294

[a]: Data excerpted from pages 127-128 of the report and rounded to whole number.

3. Dose selection, diet preparation, and diet analysis

The report states that the dose levels were selected on the basis of the results of a 28-day oral toxicity study (MRID 48119942), in which 1000, 10000, and 40000 ppm were tested. At 1000 ppm compound- related effects such as body weight decrease and clinical chemistry changes were found.

The test substance was added to the diet (amount corrected for purity) and thoroughly mixed for 5 minutes. The premix was then added to additional diet and blended for 10 minutes in a twin shell blender. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. Samples of the test diets were collected and analyzed for stability, homogeneity and concentration using HPLC; stability and homogeneity samples were taken once at the beginning of the study; concentration samples were taken once at the beginning of the study and monthly thereafter.

The analytical results showed that the test substance was at target concentrations (average 91.9 ± 8.3% of nominal), except for the 30 ppm diet during week 9, which was slightly below the acceptable range. The test substance was mixed homogeneously (mean measured values within ±12% of target concentration), and was stable in the feed for up to 14 days at room temperature (mean of stability samples at 88.3 to 102.0% of target concentration).

4. Statistics: Table 2 shows statistical methods used for analyzing the study results.

Table 2. Statistics Methods	
Endpoints	**Type of analysis**
Body weights Weekly body weight Weekly body weight change Total body weight change Food consumption Weekly food consumption Total food consumption Haematology (except leukocyte counts) Coagulation Clinical chemistry Organ weights Absolute weights Relative to body and brain weights	Group pair-wise comparisons (Levene's/ANOVA-Dunnett's/Welch's)
Leukocyte counts Total leukocyte counts Differential leukocyte counts	Log transformation/group pair-wise comparisons
Food efficiency Weekly food efficiency Total food efficiency Urinalysis Urine volume pH Osmolality	Rank transformation with Dunnett's Test

C. METHODS

1. Observations

   Animals were observed twice a day for mortality, morbidity, injury, and the availability of food and water. Detailed examinations for clinical signs of toxicity were conducted predose and once daily. Neurobehavioral observations were conducted weekly. These observations, used explicitly defined scales where possible, examined changes in the level of activity, gait, posture, altered strength, and response to handling as well as the presence of clonic or tonic movements, steriotypies (*e.g.*, excessive grooming, repetitive circling) or bizarre behaviour (*e.g.*, self-mutilation).

2. Body weights

   All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (hematology, clinical chemistry, urinalysis)

Blood and urine samples were collected from all animals twice pretest (1 week apart), and on Weeks 4, 8, and 12. Animals were fasted overnight prior to sample collection. Hematology, clinical chemistry, coagulation, bone marrow smears and urine analysis were all preformed on the samples (Table 3.). Blood samples (approximately 2 mL) were collected from all surviving animals *via* the jugular vein for determination of the plasma concentrations of the test article and metabolites. Samples were collected 2 hours after food removal on Day 57 (Week 9). The animals were not fasted prior to blood collection.

Table 3. Clinical pathology parameters evaluated

Hematology	
Erythrocyte count	Mean corpuscular haemoglobin concentration
Hemoglobin	Leukocyte count (total and differential)
Hematocrit	Reticulocytes (absolute and %)
Mean corpuscular haemoglobin	Platelet count
Mean corpuscular volume	Red cell distribution width (RDW)
Prothrombin time	Activated partial thromboplastin time
Clinical chemistry	
Alkaline phosphatase	Total protein
Total bilirubin	Albumin
Aspartate aminotransferase	Globulin and a/g ratio
Alanine aminotransferase	Glucose
Gamma glutamyltransferase	Total cholesterol
Sorbitol dehydrogenase	Electrolytes (Na, K, Cl)
Urea nitrogen	Calcium
Creatinine	Phosphorus
Triglycerides	
Urinalysis	
Volume	Bilirubin
pH	Ketones
Color and appearance	Blood
Protein	Urobilinogen
Glucose	Microscopic exam of sediment

6. Plasma concentration (Supplemental Report: MRID 48119947)

Blood samples (unfasted) were collected from all surviving animals 2 hours after food removal on Day 57 (Week 9) for determination of the plasma concentrations of the test article and selected metabolites. Plasma was prepared, frozen at approximately -70°C, and shipped to the Sponsor for analysis. Samples were evaluated by liquid

chromatography-mass spectroscopy (LC-MS-MS).

7. Sacrifice and pathology

All dogs were euthanized by anaesthesia with sodium pentobarbital and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 4. Organ weight/final body weight ratios were calculated. Tissues collected from animals in the control (0 ppm) and high dose (10000 ppm) groups were processed to slides and evaluated microscopically (Table 4). Livers from dogs in the 1000 ppm group and all gross lesions were also evaluated microscopically.

Table 4. Tissues and organs collected, weighed, and examined

Organ	Organs weighed	Microscopic/ histopathologic evaluation conducted
Adrenal (2)	X	X
Aorta		X
Bone with marrow (femur)		X
Bone with marrow (rib)		X
Bone with marrow (sternum)		X
Bone marrow smear (2 collected)		X
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X
Epididymis (2)	X	X
Eye including optic nerve (2)		X
Gallbladder	X	X
Esophagus		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Ovary (2)	X	X
Testis (2)*	X	X
Gross lesions		X
Heart	X	X
Pancreas		X
Peyer's patch		X
Pharynx		X
Pituitary	X	X
Kidney (2)	X	X
Larynx		X
Liver (3 sections collected; 2 examined)	X	X
Lung (2 sections examined)		X
Lymph node, mandibular (2 collected; 1 examined)		X
Lymph node, mesenteric		X
Nose (4 sections examined)		X
Mammary gland (process females only)		X
Prostate		X

Table 4. Tissues and organs collected, weighed, and examined		
Organ	**Organs weighed**	**Microscopic/ histopathologic evaluation conducted**
Salivary gland, mandibular (2 collected; 1 examined)		X
Sciatic nerve		X
Skeletal muscle, biceps femoris		X
Skin		X
Spinal cord (cervical, thoracic, and lumbar)		X
Spleen	X	X
Thymus	X	X
Thyroid/parathyroid (2)	X	X
Tongue		X
Trachea		X
Urinary bladder		X
Uterus (both horns) with cervix	X	X
Vagina		X

8. Liver biochemical evaluation

A sample of liver (weighing approximately 7 g) was obtained from each animal, snap frozen in liquid nitrogen, and stored at approximately -70°C for possible evaluation of cytochrome P450, but samples were not evaluated.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Mortality

One male dog in the 10000 ppm group (animal number 133) was found dead on Day 52. The cause of death was not determined but was considered to be possibly due to cardiac arrhythmia secondary to the cardiac and coronary artery effects that were attributed to canine juvenile polyarteritis syndrome (see Clinical signs of toxicity). All other animals survived until their scheduled termination.

2. Clinical signs of toxicity

In the 10000 ppm groups 3 males and 1 female were found to be thin. One of the thin male was found dead (as discussed in Mortality) and presented clinical sign such as hunched posture, and decreased activity 1 or 2 days prior to death. Another thin male had intermittent observations of decreased activity. The thin female showed signs of decreased activity, inappetence, and tremors noted intermittently. Histopathology examination showed all three thin males had arteritis; the study authors suggested that the vessels affected and histologic features of arteritis in these dogs were suggestive of canine juvenile polyarteritis syndrome (CJPS) which occurred spontaneously in beagle dogs.

3. Neurobehavioral observations

No treatment-related neurobehavioral findings were observed.

B. BODY WEIGHT AND BODY WEIGHT GAIN

At 10000 ppm, the final (Week 13) mean body weight and total body weight change in males were significantly reduced (18% and 85% below control, respectively) (Tables 5 and 6) . Mean body weight change in this group was below controls over most weekly intervals. Similarly, in 10000 ppm females, there were decreases in final mean body weight and body weight gain (11% and 51% below controls, respectively), but the decreases did not demonstrate statistically significance. The decreased in body weight and body weight gain in 10000 ppm females were due to 2 females and the mean body weight gain decrease was 50%. Body weight and body weight gain in other male and female groups were comparable to those of the control.

Table 5. Body weights (kg) (n=4)

	0 ppm	30 ppm	100 ppm	1000 ppm	10000 ppm
Males					
Week -1	7.08±0.87	7.30±0.42	7.48±0.43	7.53±0.89	7.77±0.40
Week 13	9.72±0.76	9.96±0.45	9.82±0.80	10.67±0.70	7.98±1.08 * (↓18%)
Females					
Week -1	5.79±0.40	5.94±0.69	5.94±0.42	5.77±0.59	5.87±0.38
Week 13	7.51±0.86	8.08±1,50	7.70±0.81	7.45±0.45	6.72±1.12(↓11%)

* Significantly different from control, p <0.05. Data excerpted from pages 89-92 of the report.

Table 6. Body weight gain (kg) (n=4)

Parameter	0 ppm	30 ppm	100 ppm	1000 ppm	10000 ppm
Males					
Overall body weight gain (Weeks -1 to 13)	2.65±0.18	2.66±0.32	2.34±0.62	3.14±0.24	0.39±1.14* (↓85%)
Females					
Overall body weight gain (Weeks -1 to 13)	1.71±0.73	2.14±0.81	1.76±0.46	1.68±0.36	0.85±0.98(↓50%)

*: Significantly different from control, p <0.01. Data excerpted from pages 99-102 of the report.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Mean weekly food consumption and food efficiency data for 10000 ppm males and females were below control over all (food consumption) or most (food efficiency) weekly intervals (Table 7). In males, the differences in both parameters were statistically significant over multiple weeks. Overall (weeks 1 to 13) mean food consumption and food efficiency in 10000 ppm males were 19% and 87% below control, respectively. Overall mean food consumption and food efficiency in 10000 ppm females were 9% and 53% below control, respectively. These differences were considered test-article related and adverse. Food

consumption and food efficiency in other male and female groups were comparable to control.

Table7. Food consumption and food efficiency (1-13 Weeks) (n=4)					
Parameter	**0 ppm**	**30 ppm**	**100 ppm**	**1000 ppm**	**10000 ppm**
Males					
Food consumption, total (g/animal/day)	281.7±40.2	291.8±22.9	276.8±11.5	303.4±12.2	228.0±32.6 (↓19%)
Food efficiency, total (%)	10.50	10.0	9.4	11.4	1.4(↓87%)
Females					
Food consumption, total (g/animal/day)	207.1±18.9	239.9±39.1	247.8±50.3	233.2±32.3	189.3±44.7 (↓9%)
Food efficiency, total (%)	8.9	9.6	7.9	8.1	4.1(↓53%)

Data excerpted from pages 109-112 and 119-122 of the report.

D. OPHTHALMOLOGICAL EXAMINATIONS

No statistically significant or biologically significant changes in the incidences of ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Hematology

There were increases in leukocytes, neutrophils, and monocytes in10000 ppm males and females at week 8 and in 10000 ppm males at week 12 relative to the controls (Table 8). However, the increase was not statistically significant. Other hematological parameters were not affected by treatment.

Table 8. Summary data for leukocytes, neutrophils, and monocytes

Dose group	**Leukocytes (10^3/µL)**	**Neutrophils (10^3/µL)**	**Monocytes (10^3/µL)**
Week 8	**Males**		
0 ppm (n=4)	10.00	6.168	0.475
10000 ppm (n=3)	13.10 (↑31%)	9.117 (↑48%)	0.907(↑91%)
	Females		
0 ppm (n=4)	8.83	5.485	0.475
10000 ppm (n=4)	15.83 (↑79%)	11.158 (↑103%)	0.893 (↑88%)
Week 12	**Males**		
0 ppm (n=4)	10.00	6.168	0.475
10000 ppm (n=3)	17.63 (↑76%)	12.757 (↑107%)	1.037 (↑118%)
	Females		

0 ppm (n=4)	11.10	6.800	0.583
10000 ppm (n=3)	11.90	6.955	0.503

*: Statistically significant ($p<0.05$). Data excerpted from pages 130 – 153 of the study report.

2. Clinical chemistry

Statistically significant decreases in albumin (13%- 39%), total protein (8%-17%) and cholesterol concentrations (16%-41%) were observed in 1000 ppm or above males and females at various time (Table 8). Alkaline phosphatase was also significantly increased in both males and females at or greater than 1000 ppm. An increased in alanine transferase was seen in female dogs at 1000 ppm or greater (9%-284%). When these changes were considered together, they collectively indicated that the liver was adversely affected by cyantraniliprole at 1000 ppm or above. It should be noted that decreased albumin and cholesterol level were also seen at similar dose levels in the 28 day feeding study (MRID 48119942). No adverse test article-related effects on any clinical pathology parameter were observed in the 30 or 100 ppm groups.

Table 9. Clinical chemistry evaluation (n=4) [a]

ppm	0	30	100	1000	10000
Males					
Albumin (g/dL)					
Week 4	2.83±0.49	2.95±0.06	2.90±0.09	2.45±0.13(↓13%)	2.25±0.06*(↓21%)
Week 8	2.58±0.34	2.55±0.10	2.50±0.08	2.03±0.13**(↓21%)	1.63±0.12**(↓37%)
Week 12	3.00±0.34	2.98±0.10	2.88±0.05	2.23±0.10**(↓26%)	1.83±0.31**(↓39%)
Alkaline phosphatase (U/L)					
Week 4	74.3±12.0	83.5±11.7	141.3±41.3	233.0±74.6**(↑214%)	189.3±93.9*(↑155%)
Week 8	76.5±14.4	102.0±35.0	169.8±78.6	276.5±89.2(↑261%)	426.7±362.3*(↑458%)
Week 12	65.5±12.0	93.5±38.9	138.3±60.1	293.3±115.2*(↑348%)	363.7±246.2*(↑455%)
Cholesterol (mg/dL)					
Week 4	170.0±37.6	185.3±15.6	175.8±39.7	142.5±29.4 (↓16%)	100.3±14.1*(↓41%)
Week 8	168.3±41.7	184.8±15.7	161.3±39.7	130.5±32.2(↓22%)	108.0±49.4(↓36%)
Week 12	162.8±48.0	173.3±18.4	154.5±36.8	127.5±36.4(↓22 %)	125.3±72.2(↓23%)
Total protein (g/dL)					
Week 4	5.48±0.40	5.50±0.25	5.43±0.17	5.00±0.22(↓9%)	4.83±0.25(↓12%)*
Week 8	5.33±0.15	5.25±0.17	5.20±0.25	4.55±0.33**(↓15%)	4.63±0.38(↓13%)*
Week 12	5.78±0.10	5.63±0.28	5.60±0.26	4.83±0.33**(↓17%)	5.07±0.65(↓12%)
Females					
ALT (Units/L)					
Week 4	22.0±4.8	27.3±5.7	28.3±3.4	24.0±7.1(↑9%)	36.0±2.2**(↑64%)
Week 8	21.3±2.6	25.5±3.7	26.3±4.0	28.0±7.5(↑31%)	42.5±23.5(↑99%)
Week 12	22.8±5.0	26.0±6.6	27.3±27.3	31.0±12.8(↑36%)	87.5±30.6**(↑284%)
Albumin (g/dL)					

Table 9. Clinical chemistry evaluation (n=4) [a]					
ppm	**0**	**30**	**100**	**1000**	**10000**
Week 4	2.80±0.29	2.85±0.27	2.65±0.39	2.38±0.19(↓15%)	2.20±0.08*(↓21 %)
Week 8	2.55±0.27	2.60±0.14	2.40±0.18	1.98±0.13**(↓22%)	1.58±0.25**(↓38%)
Week 12	2.95±0.30	2.90±0.12	2.78±0.17	2.30±0.00**(↓28%)	1.80±0.20**(↓39%)
Alkaline phosphatase (U/L)					
Week 4	91.5±12.6	113.0±33.2	160.8±39.7	266.3±57.1*(↑191%)	257.3±143.3*(↑181%)
Week 8	90.5±19.5	120.316.9±	179.0±40.4	307.0±104.5(↑239%)	483.5±321.6**(↑434%)
Week 12	77.3±13.4	98.8±21.6	142.0±22.7	259.3±98.7**(↑235%)	357.8±85.9**(↑363%)
Cholesterol (mg/dL)					
Week 4	154.8±30.6	150.3±12.4	179.8±27.0	156.0±23.9	97.8±43.6*(↓37%)
Week 8	155.5±23.5	147.8±18.9	173.3±27.0	134.3±15.4(↓14%)	114.3±72.8(↓26%)
Week 12	161.0±28.2	146.3±20.6	155.3±14.0	143.8±17.8(↓11%)	90.8±46.4**(↓44%)
Total protein (g/dL)					
Week 4	5.08±0.30	5.33±0.13	5.38±0.25	5.13±0.30	4.63±0.05*(↓9%)
Week 8	4.98±0.24	5.08±0.05	5.33±0.42	4.60±0.25(↓8%)	4.45±0.38(↓11%)
Week 12	5.33±0.26	5.43±0.13	5.58±0.38	4.93±0.32(↓8%)	4.50±0.12(↓16%)**

* Significantly different from control, p <0.05.
** Significantly different from control, p <0.01.
Data excerpted from pages 164-183 of the report.
[a]: n=3 for 10000 ppm males at weeks 8 & 12.
Note: ALT = Alanine aminotransferase

3. Urinalysis

There were no adverse changes in urine parameters in male or female dogs.

F. PLASMA CONCENTRATIONS

Blood samples were collected from all treatment groups and analyzed; the results showed that the most abundant analyte in plasma was parent cyantraniliprole (HGW86) which was present at comparable concentrations in both males and females at the same dietary concentrations except at 30 ppm (Table 10). At 30 ppm plasma concentration of HGW86 was approximately 40% more in the female than that in males. This observation is consistent with results found in the metabolism study (MRID 4819949; DuPont-16995), which demonstrates a greater internal in female rats than in male rats. The concentration of any of the metabolites (IN-J9Z38, IN-MYX98, IN-MLA84, and IN-N7B69) was substantially less that that of the parent compound.

Table 10. Plasama concentration (ng/ml) of parent cyantraniliprole (DPX-HDW86) and major metabolites at Day 57.				
ppm	**30**	**100**	**1000**	**10,000**
Males				
HGW86	1743±1110	16806±6046	30963±6988	51900±6597
IN-J9Z38	181±61	562±185	1486±97	2668±1205
IN-MYX98	256±93	718±159	8713±2250	18717±7877

Table 10. Plasama concentration (ng/ml) of parent cyantraniliprole (DPX-HDW86) and major metabolites at Day 57.				
ppm	**30**	**100**	**1000**	**10,000**
IN-MLA84	32±7	83±82	217±56	359±183
IN-N7B69	252±154	798±427	11676±8501	8558±8568
Females				
HGW86	2424±1087	20600±10772	28383±18056	51263±26885
IN-J9Z38	96±28	661±491	1176±565	2043±488
IN-MYX98	77±23	1021±1123	1766±1070	4048±895
IN-MLA84	<LOQ	105±93	158±87	567±255
IN-N7B69	81±21	946±1402	2543±1992	3801±1978

Data excerpted from page 14 of the report.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Increases in the absolute and relative liver weights were observed in males and females at 100, 1000, and 10000 ppm (Table 11). No correlative microscopic abnormalities were observed and the increased liver weights are likely due to induction of cytochrome P450 metabolic enzymes, observed at ≥1000 ppm in a previous 28-day feeding study in male and female dogs (DuPont-15456; MRID 48119942). The liver weight increase at 100 ppm was associated with marginal increase of ALP or other parameters. In the absence of correlative clinical or microscopic pathology findings, the liver weight increase at 100 ppm or below was considered to be adaptive and non-adverse. Occasional statistically significant differences (from control) were observed in other organ weights but were considered to be spurious as there was a no dose response or correlative microscopic changes.

Table 11. Organ weights (terminal)

Parameter	0 ppm	30ppm	100 ppm	1000 ppm	10000 ppm
Males					
Liver w/gallbladder: Absolute weight (g)	278.2±21.4	318.2±32.5	346.6±20.6 (↑25%)	**439.4±35.7** (↑56%)**	**359.9±92.6 (↑29%)**
Relative[a] weight (%)	3.1	3.5	3.8	**4.6****	**4.9 ****
Relative to to brain (%)	3.6	4.3	4.4	**6.1 ****	**5.2**

Table 11. Organ weights (terminal)

Parameter	0 ppm	30ppm	100 ppm	1000 ppm	10000 ppm
Kidneys:					
Absolute weight (g)	42.11±4.01	41.01±3.88	43.81±3.00	47.92±1.38	51.65±10.71
Relative[a] weight (%)	0.48	0.46	0.49	0.50	0.72*
Relative to to brain (%)	0.55	0.56	0.56	0.63	0.75*
Spleen:					
Absolute weight (g)	34.61±7.69	47.96±11.34	48.45±21.70	38.30±7.39	50.50±5.70
Relative[a] weight (%)	0.39	0.54	0.53	0.41	0.69
Relative to to brain (%)	0.46	0.64	0.62	0.53	0.73
Thyroid/parathyroid:					
Absolute weight (g)	0.73±0.27	0.74±0.10	0.90±0.09	0.71±0.27	0.76±0.04
Relative[a] weight (%)	0.01	0.01	0.01	0.01	0.01
Relative to to brain (%)	0.01	0.01	0.01	0.01	0.01
Thymus					
Absolute weight (g)	8.34±2.72	5.92±2.38	4.70±1.21	5.89±1.97	4.25±0.90
Relative[a] weight (%)	0.10	0.06	0.05	0.06	0.06
Relative to to brain (%)	0.11	0.08	0.06	0.08	0.06
Females					
Liver/Gallbladder:					
Absolute weight (g)	208.1±17.0	257.9±48.9 (↑24%)	262.9±28.0 (↑26 %)	**325.5±42.9** (↑66%)**	**359.5±97** (↑73%)**
Relative[a] weight (%)	3.1	3.5	3.9	**4.8 ****	**6.0 ****
Relative to brain weight (%)	3.0	3.4	3.8	**4.5 ****	**5.4 ****
Kidneys:					
Absolute weight (g)	31.32±3.30	35.54±4.42	36.27±5.84	33.73±53.16	38.24±8.89
Relative[a] weight (%)	0.48	0.49	0.53	0.50	0.65
Relative to to brain (%)	0.46	0.48	0.52	0.47	0.58
Spleen:					
Absolute weight (g)	36.72±2.85	39.57±3.17	52.62±12.86	41.67±10.36	39.49±11.78
Relative[a] weight (%)	0.54	0.55	0.76	0.62	0.66
Relative to to brain (%)	0.53	0.53	0.75	0.58	0.60
Thyroid/parathyroid:					
Absolute weight (g)	0.49±0.08	0.65±0.21	0.69±0.15	0.62±0.14	0.65±0.07
Relative[a] weight (%)	0.01	0.01	0.01	0.01	0.01*
Relative to to brain (%)	0.01	0.01	0.01	0.01	0.01
Thymus					
Absolute weight (g)	7.17±3.41	9.35±5.04	4.39±1.71	5.38±1.32	4.54±3.35
Relative[a] weight (%)	0.10	0.13	0.06	0.08	0.07
Relative to to brain (%)	0.10	0.13	0.06	0.07	0.07

[a] Relative weight is defined as the organ to body weight ratio. Data excerpted from pages 195-210 of the study report.

* Significantly different from control, $p < 0.05$.

** Significantly different from control, $p < 0.01$

Note: Bolded values were interpreted to be test substance-related increases, as compared to control values.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy.

Test substance-related liver microscopic pathology was observed at 10000 ppm (Table 12). Minimal bile duct hyperplasia was observed in the liver of 3/4 males and 3/4 females at 10000 ppm. Portal areas were hypercellular with oval–like cells and additional small branching bile ductules present. A few mixed mononuclear inflammatory cells were occasionally seen in these areas. Adjacent hepatic plates were normal. Examination of liver sections from males and females at 1000 ppm revealed no bile duct hyperplasia. Other liver changes were observed but were considered secondary to systemic arteritis in two dogs. One male at 10000 ppm had minimal individual hepatocyte necrosis and sinusoidal leukocytosis. Occasional individual liver cells, in no particular zonal pattern, were rounded and deeply eosinophilic with pyknotic nuclei. Neutrophils surrounded these dead hepatocytes and were more numerous in sinusoids throughout the liver sections. One female at 10000 ppm had mild individual hepatocyte necrosis, hypertrophy of Kupffer cells throughout the sections, and mild granulomatous inflammation. Individual groups of two to four liver cells, in no particular zonal pattern, were rounded and deeply eosinophilic with pyknotic nuclei. Macrophages surrounded these dead hepatocytes in some instances and in other cases macrophages were focally numerous, but the dead hepatocytes were no longer visible.

Table 12. Histopathology Findings

Observation	0 ppm	100 ppm	1000 ppm	10000 ppm
Males (n=4)				
Arteritis	0			1
Liver – Hyperplasia of bile duct	0		0	3
Liver – Leukocytosis, sinusoidal	0		0	1
Liver – Individual hepatocyte necrosis	0		0	1
Thymus - Arteritis	0		0	2
Thymus - Atrophy	0		0	2
Thyroid - Arteritis	0			1
Females (n=4)				
Liver – Hyperplasia of bile duct	0	0	0	3
Liver – Kupffer cell hypertrophy/hyperplasia	0	0	0	1
Liver – Individual hepatocyte necrosis	0	0	0	1
Liver – focal necrosis	0	0	0	2
Thymus - Atrophy	0	0	0	1

Data excerpted from pages 212 – 229 of the report.

Compound-related increase in the incidence of arteritis was present in 3 animals (2 males; 1 female) at 10000 ppm. As previous discussed in the Data Evaluation Report (DER) of the 28-day oral toxicity study in dog (MRID 48119942), the study author claimed that the microscopic findings in the dogs with arteritis were compatible with spontaneous canine juvenile polyarteritis syndrome (CJPS), as distinguished from test article-related arteritis, and these changes were not considered to be test article related. However, CJPS was thought to be mediated by the immune system, but cyantraniliprole did not show any effect on the immune system in two immunotoxicity studies in rats

and mice (MRID 48119971 & MRID 48119972). Based on the currently available data, the increase in the incidence of arteritis seen in this study was compound-related.

III. CONCLUSION

The no-observed-adverse-effect level (NOAEL) for cyantraniliprole in male and female dogs was 100 ppm (3 mg/kg bw/day in males and females). The LOAEL was 1000 ppm (32 and 34 mg/kg bw/day in males and females, respectively) based on a collection of treatment-related effects indicative of hepatotoxicity. The effects included decreases in total protein, albumin, and cholesterol in males and females; increases in alkaline phosphatase in males and females, and increases in alanine aminotransferase in females, and increased liver weights in males and females.

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.3150) for a 90-oral toxicity study in dogs.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.1.1 Metabolism study – rats

IIA 5.1.1/01 ADME in male a d female rats

Report: Gannon, S.A. (2010); ^{14}C-DPX-HGW86: Absorption, distribution, metabolism and excretion in male and female rats. DuPont Haskell Laboratories, Newark, Delaware, USA.,DuPont-16995, Revision No. 1. MRID 48119949. Unpublished.

Guidelines: U.S. EPA 870.7485 (1998)
OECD Section 4 Prt (417; 1984) and Annex to Directive 87/302/EEC, Part B, Toxicokinetic (1987)
JMAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance and Data Confidentiality statements were presented in the report.

Executive summary:

In a metabolism study (MRID 48119949), groups of 4 male and 4 female Sprague-Dawley Crl:CD®(SD) rats were administered by gavage a single dose of either 10 or 150 mg/kg bw (low- and high-dose, respectively) of either [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]- cyantraniliprole. All rats received the appropriate levels of radioactivity (μCi/animal) at the targeted dose levels; the mean radioactive dose ranged from 23.2 to 49.7 μCi for male rats and 16.8 to 34.9 μCi for female rats.

The study examined absorption, distribution, metabolism, and elimination of cyantraniliprole. Experiments were performed to explore the following parameter: pharmacokinetic behaviour of radioactive residues in plasma and red blood cells, the disposition and material balance of total ^{14}C residues among tissues and excreta, the percentage and concentration of ^{14}C residues in tissues at selected times after dosing (T_{max}, and terminal sacrifice), and the elimination of ^{14}C residues in bile. The profile of metabolites was characterized in urine, faeces and bile.

The data indicated that cyantraniliprole was absorbed readily either at low (10 mg/kg bw) or high (150 mg/kg bw) dose with oral dosing. The majority of the absorption occurred during the first 48 hours, and the peak plasma concentration was reached at approximately 2 hour after dosing irrespective of the position of label, sex of the test animal, and dose level. Both species of radiolabeled cyantraniliprole exhibited very similar kinetics (male low dose half-lives: CN $T_{1/2}$ = 42 hrs; PC $T_{1/2}$ = 54 hrs; female low dose half-lives: CN $T_{1/2}$ = 129 hrs; PC $T_{1/2}$ = 117 hrs; male high dose half-lives: CN $T_{1/2}$ = 62 hrs; PC $T_{1/2}$ = 55 hrs; females high dose half-lives: CN $T_{1/2}$ = 65 hrs; PC $T_{1/2}$ = 80 hs). The values of C_{max} and area-under-the-curve (AUC) demonstrated a greater internal dose in female rats than in male rats. With dose-normalized AUC, the data suggested a decrease in absorption at high dose in comparison to the low dose.

The distribution of ^{14}C residues was evaluated as the percentage of the administered dose, concentration of ^{14}C equivalents per gram of tissue, and tissue:plasma concentration ratios at T_{max}, T_{max}/2, and terminal sacrifice after single oral dose administration. There was no

appreciable difference between dosing with the [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole label in terms of tissue distribution. The majority of the dose was initially associated with the GI tract contents and subsequently showed uptake and distribution to all tissues. The percent recovery and tissue concentration data showed that female rats retained a greater proportion of ^{14}C residues than male rats. The declines of the plasma and tissue ^{14}C residue concentrations at high and low doses were generally similar. These observations were consistent with the shorter elimination half-life in male rats compared with female rats. The tissue:plasma concentration ratios were less than or equal to 1 by 168 hours after dose administration. Many of the tissue:plasma concentration ratios were below 1 at the $T_{max}/2$ time point as well.

The bile cannulation study showed that there was no appreciable difference between the two forms of radiolabeled cyantraniliprole. The mean total recovery for the absorbed and unabsorbed (faeces, cage wash, and GI tract contents) radioactivity was 89.0 to 101.6% of the dose. Absorption was considerably higher at the 10 mg/kg bw dose level in which 75.8 to 80.4% and 62.6 to 74.9% of the dose was measured for male and female rats, respectively, compared with 38.8 to 40.0% and 31.4 to 32.2% of the dose for male and female rats administered cyantraniliprole at 150 mg/kg bw, respectively.

Metabolism results suggested that cyantraniliprole was readily hydroxylated to form IN-N7B69 and IN-MYX98. IN-N7B69 was further metabolized to a glucuronide. Cyantraniliprole also underwent ring closure to generate IN-J9Z38 which was hydroxylated to form IN-NBC94, its carboxylic acid, and its glucuronide conjugate. IN-MYX98 was also metabolized to the closed-ring metabolite IN-MLA84, which, like IN-NBC94, was further oxidized to a hydroxylated metabolite, a carboxylic acid, and the glucuronide of the hydroxyl metabolite. Further, the hydroxylated metabolite IN-MYX98 could be N-dealkylated to form IN-HGW87 as well as being hydroxylated a second time to form bis-hydroxy-HGW86. Cyantraniliprole could also be hydroxylated on the pyridine ring, followed by a ring closure analogous to the conversion of cyantraniliprole to IN-J9Z38. Cyantraniliprole could also be N-dealkylated and cleaved at the carbonyl bridge to form IN-DBC80. The metabolites which were found to be greater than 5% of the administered dose were bis-hyroxy-cyantraniliprole, IN-N7B69, IN-MYX98, INDBC80, and the parent compound.

There was very little difference in elimination between rats administered [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole. Rats given a single 10 mg/kg bw dose of either species of cyantraniliprole excreted a greater percentage of the dose in urine (22.0 to 34.6%) than rats dosed with 150 mg/kg bw (11.8 to 14.8%). For both dose levels and labels, the majority of the dose was excreted by 24 to 48 hours after administration. The percent recovery in rats at the 10 mg/kg dose level was 22.0 to 34.6% for urine, 46.8 to 61.6% for faeces, and 1.1 to 5.3% for tissues. Lower absorption from the GI tract occurred in rats given the 150 mg/kg bw dose as indicated by the lower percentage of the dose in tissues (0.25 to 2.5%), and urine (11.8 to 14.8%) and the greater percentage of the dose excreted in the faeces (77.6 to 80.1%). For all groups in which material balance was measured, the mean percentage for total recovery by 7 days after dosing ranged from 88.3 to 96.5%. The data also indicate that there was no appreciable tendency for bioaccumulation to occur for cyantraniliprole.

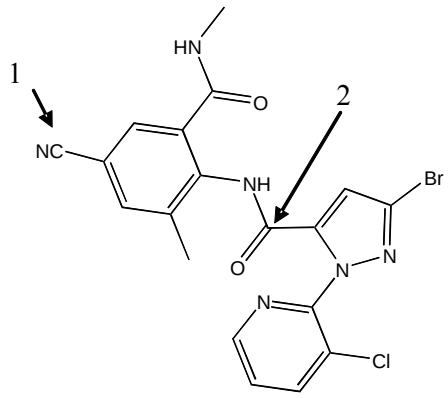
This study is fully reliable (acceptable/guideline) and satisfies the study requirements of a metabolism study (U.S. EPA 870.7485; OECD Section 4 Part 417 and Annex to Directive 87/302/EEC, Part B, Toxicokinetic; JMAFF 12 Nousan 8147).

1I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: HGW86-141
   Purity: 93.4%
   Description: Solid, powder
   CAS #: 736994-63-1
   Stability of test compound: Not determined.
2. Radiolabeled test material: ^{14}C-cyantraniliprole technical
   Lot/Batch #: [Cyano-^{14}C]-cyantraniliprole, abbreviated as [CN-^{14}C]-cyantraniliprole
   Lot #: 3503-242
   [Pyrazol-Carbonyl-^{14}C]-cyantraniliprole abbreviated as [PC-^{14}C]-cyantraniliprole
   Lot #: 3503-247

   Chemical structure /positions of radiolabels:
   1. [CN-^{14}C]-cyantraniliprole
   2. PC-^{14}C]-cyantraniliprole



   Radio-chemical purity: [CN-^{14}C]-cyantraniliprole: 99%
   [PC-^{14}C]-cyantraniliprole: 99%
   Specific activity: [CN-^{14}C]-cyantraniliprole: 16.95 µCi/mg
   [PC-^{14}C]-cyantraniliprole: 15.33 µCi/mg
   Description:
   Stability of test compound: The positions of the radiolabels are considered to be stable.
3. Vehicle and/or positive control: Polyethylene glycol (PEG 400)
4. Test animals
   Species: Rat

Strain:	Crl:CD®(SD)
Age at dosing:	At least 8 weeks old
Weight at dosing:	175.6–342.3 (male rats), 151.3–205.8 (female rats)
Source:	Charles River Laboratories, Raleigh, NC Jugular vein cannulated rats were obtained from Charles River Laboratories (Raleigh, North Carolina, U.S.A.). Bile duct cannulated rats were obtained from Taconic (Germantown, New York, U.S.A.).
Acclimation period:	Minimum of 6 days quarantine in stainless steel suspension cages except for cannulated rats, which had minimum of 3 days quarantine.
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum.* Animals were fasted before dosing with test substance. Food was returned approximately 2 hours post-dose. The feed was switched to dustless pellets (Product #F0165, Bio-Serv, Frenchtown, New York, U.S.A.) when animals without jugular vein or bile duct cannulas were housed in glass metabolism units. Access to feed for cannulated animals in the glass metabolism units was maintained using Certified Rodent LabDiet® 5002 and a feeder suspended from the inside wall of the cage.
Water:	Tap water, *ad libitum*
Housing:	Rats were housed separately in stainless steel, wire-mesh cages suspended above cage boards.

5. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

6. Preparation of the dosing solutions

Rats were dosed with approximately 30 µCi/animal of either [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole. The ^{14}C-cyantraniliprole radiolabels were diluted with cyantraniliprole technical to the appropriate specific activity for each dose level. The dose vehicle (PEG 400) was added and mixed to a clear solution or homogeneous suspension. Dose level, volume, and radiochemical data are presented in Table 1. The test compound was administered by single oral gavage dose.

Table 1. Dose Levels, Volumes, and Radiochemical Data

Dose Level (mg/kg bw)	Dose Volume (mL/kg bw)	Radiochemical Dose (µCi/kg bw)	Specific Activity in Dose (µCi/mg)	Chemical Concentration (mg/mL)	Radiochemical Concentration (µCi/mL)
10	4	120	12	2.5	30
150	4	120	0.8	37.5	30

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   17-August-2005 to 06-December-2006

2. Pilot study

   The dose levels were based on the results of a pilot study, which showed plasma levels after 150 mg/kg or 450 mg/kg were equivalent. Since saturation occurred at 150 mg/kg, this level was selected as the high dose for this study.

3. Main study

   Animals were assigned to test groups according to Table 22.

Table 2. Study design

Experiment[a]	Dose level (mg/kg bw[b])	Label[c]	Number of animals		Time of sacrifice (hours)	Samples
			Male	Female		
Pharmacokinetics	10	CN	4	4	168	Plasma, red blood cells
		PC	4	4		
	150	CN	4	4		
		PC	4	4		
Volatiles	10	CN	1	1	≤168	Exhaled volatile & CO_2, urine, faeces
		PC	1	1		
Mass balance and tissue distribution (terminal)	0	PEG 400	1	1	168	Urine, faeces, tissues, carcass
	10	CN	4	4	168	Urine, faeces, tissues, carcass, cage wash, feed residue metabolite profile in excreta
		PC	4	4		
	150	CN	4	4		
		PC	4	4		
Tissue distribution (T_{max})	10	CN	4	4	—[a]	Tissues, carcass
		PC	4	4		
	150	CN	4	4		
		PC	4	4		
Tissue distribution ($T_{max}/2$)	10	CN	4	4	—[a]	Tissues, carcass
		PC	4	4		
	150	CN	4	4		

Experiment[a]	Dose level (mg/kg bw[b])	Label[c]	Number of animals		Time of sacrifice (hours)	Samples
			Male	Female		
		PC	4	4		
Biliary elimination	10	CN	4	4	48	Bile, urine, faeces, GIT[d], carcass Metabolite profile in bile
		PC	4	4		
	150	CN	4	4		
		PC	4	4		

a T_{max} and $T_{max}/2$ was determined experimentally based on pharmacokinetic data.
T_{max} = Time at maximum plasma concentration (C_{max})
$T_{max}/2$ = Time at half of plasma C_{max}

b bw = Body weight

c CN = [CN-^{14}C]-cyantraniliprole
PC = [PC-^{14}C]-cyantraniliprole.

d GIT = Gastrointestinal tract tissue and contents

4. Plasma Kinetics Following 10 and 150 mg/kg bw Oral Dosing

Four male and four female rats with surgically implanted jugular vein cannulas were allowed to recover for at least 3 days after surgery before dosing. [PC-^{14}C]DPX-HGW86 was administered to the rats by oral gavage at a dose level of approximately 10 mg/kg bw. This experiment was repeated at 150 mg/kg bw. Both dose levels were then repeated with the CN label. Following dosing, serial blood samples were removed from the jugular vein cannula. In some instances, whole blood was collected from the orbital sinus when a jugular vein cannula lost patency. Samples were collected predose, and at 15 and 30 minutes, 1, 2, 4, 8, 12, and 24 hours post dose, and at 24-hour intervals thereafter up to 120 hours.

5. Tissue Distribution at Tmax (10 and 150 mg/kg bw)

Tissues and carcass were analyzed to determine ^{14}C tissue distribution. The following tissues were collected at sacrifice from the test jugular vien cannulated test animals :

Blood (plasma & RBC)a	Fat	Liver
Kidney	Muscle	Heart
Lung	Testes	Ovaries
Uterus	Bone and bone marrowa	Brain
Spleen	Adrenals	Pituitary
G.I. tract and contentsa	Pancreas	Skin sample
Thyroid	Thymus	Bladderb

a Analyzed separately for ^{14}C content
b Urine in bladder at sacrifice was added to the terminal urine sample.

6. Biliary Elimination Following 10 and 150 mg/kg bw Oral Dosing

Four male and four female rats with surgically implanted bile duct cannulas were obtained from the vendor and allowed to recover for at least 3 days after surgery before dosing. [PC-

^{14}C]DPX-HGW86 was administered to the rats by oral gavage at a dose level of approximately 10 mg/kg bw. This experiment was repeated at 150 mg/kg bw. Both the dose levels were repeated with the CN label.

Urine, feces and bile were collected on dry ice at intervals of 0-6, 6-12, 12-24, and 24-48 hours post-dose. At sacrifice, the blood that was collected by exsanguination was frozen, weighed as part of the carcass, and processed with the carcass. Urine, feces, bile, gastrointestinal tract contents and tissue (analyzed separately), carcass and cage wash were analyzed to determine the absorbed dose which was calculated as the sum of radioactivity in urine, bile, carcass, and gastrointestinal tissue (minus contents).

7. Quantitation of Radioactive Residues

Radioactive residues were measured in exhaled volatiles and $^{14}CO_2$, plasma, urine, bile, feces, tissues and red blood cells, cage rinses, feed residues, and carcass using liquid scintillation counting (LSC), liquid chromatography-mass spectrometry.

8. Quantification and Identification of Metabolites

Liquid chromatography/mass spectrometry methods were employed for identification and quantification of the metabolites.

Urine
The urine samples were pooled across the individual animals for a given collection interval to obtain a kinetic representation at Day 1, Day 7, and Day 14. Individual components greater than 0.5% of the administered dose are labeled on the radio-chromatograms. Cyantraniliprole and its metabolites were identified by comparing LC/MS full scan spectra, daughter ion spectra, and retention times for the corresponding reference standards. Where reference standards were not available, tentative structures were assigned based on mass spectral data.

Feces
The faeces samples were pooled across the individual animals for a given collection interval to obtain a kinetic representation at Day 1, Day 7, and Day 14. Cyantraniliprole and its metabolites were identified by comparing LC/MS full scan spectra, daughter ion spectra, and retention times for the corresponding reference standards. Where reference standards were not available, tentative structures were assigned based on mass spectral data.

Plasma
Plasma samples were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Selected plasma samples were analyzed for parent compound and metabolites by LC-MS.

Tissues and Red Blood Cells
Tissues and red blood cells were homogenized and aliquots combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total radioactivity present in the tissue.

Cage Rinses
Aliquots of cage rinses were analyzed in triplicate for ^{14}C by LSC.

II. RESULTS AND DISCUSSION

A. STORAGE STABILITY

The test substance and major metabolites (*i.e.*, those found to be greater than 5% of the dose) were found to be stable in urine and faeces extracts over a time interval of one month.

B. MAIN STUDY

Clinical Observation

Four rats from the Biliary Elimination group were found dead, and they appeared to have been replaced because even the individual animal data did not identify which animal died and when the animals died. The study report, however, attributed the deaths to the quality of the bile cannulation surgery, not to the test substance, based on the fact that other rats at this same dose level within this study did not die, nor did rats at a much higher dose level in a similar study in bile cannulated rats.

Absorption

The pharmacokinetic results indicated that absorption occurred readily following low (10 mg/kg bw) and high (150 mg/kg bw) dose single oral gavage administration. The peak plasma concentration was reached approximately 2 hours following either Cyano-^{14}C] DPX-HGW86 or [pyrazole carbonyl-^{14}C] DPX-HGW86 dosing (Tables 3& 4).

Table 3. Concentration (µg equiv/g) in plasma following a 10 or 150 mg/kg bw single dose of [CN-^{14}C] DPX-HGW86 (Table excerpted from page 44 of the report).

	10 mg/kg bw				150 mg/kg bw			
	Male		Female		Male		Female	
Hour	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.25	1.51	0.88	3.42	0.52	25.82	16.67	26.83	6.06
0.5	3.13	1.30	8.91	1.51	34.46	18.11	38.35	15.17
1	5.26	2.45	10.42	1.10	39.89	15.93	38.43	12.88
2	6.31	2.08	11.28	1.31	40.00	14.57	44.19	16.01
4	2.60	0.49	5.82	0.88	20.78	7.60	38.38	10.42
8	2.14	0.59	4.52	1.25	13.36	4.29	26.42	9.83
12	2.42	0.82	3.53	0.50	14.49	5.45	23.87	7.19
24	1.92	1.02	2.95	0.65	15.42	6.64	28.77	6.77
48	1.30	0.93	2.35	0.60	12.19	5.06	22.49	6.97
72	0.86	0.75	2.11	0.49	8.40	3.38	16.17[a]	8.43
96	0.65	0.64	1.82	0.51	5.91	1.64	11.43	8.11
120	0.72[a]	0.53	1.47	0.42	4.51	1.02	9.33	8.12
144	0.54[a]	0.44	1.44	0.39	3.59	0.81	7.43	6.89
168	0.29[a]	0.15	1.13	0.34	2.78	0.42	6.04	6.35

a n = 3

Table 4. Concentration (µg equiv/g) in plasma following a 10 or 150 mg/kg bw single dose of [PC-^{14}C] DPX-HGW86 (Table excerpted from page 44 of the report)

	10 mg/kg bw				150 mg/kg bw			
	Male		Female		Male		Female	
Hour	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.25	1.11	0.68	3.09	1.40	33.03	4.15	25.83	9.60
0.5	2.34	1.23	6.21	3.27	40.31	12.04	35.71	10.68
1	3.51	0.73	7.91	2.22	37.81	7.95	45.95	20.47
2	4.45	0.62	10.11	0.57	39.17	7.95	47.53	11.12
4	3.92	0.94	5.15	1.50	23.96	12.22	40.37	16.11
8	2.65	0.46	3.91	0.76	14.69	7.47	29.33	10.96
12	2.17	1.06	3.60	0.36	17.36	10.34	28.87	7.88
24	1.97	0.85	3.30	0.78	17.25	10.02	34.35	9.24
48	1.47	0.84	2.54	0.56	10.52	6.76	35.34	10.31
72	1.03	0.59	2.27	0.17	9.98	7.76	24.96	8.21
96	1.10[a]	0.89	2.00[a]	0.58	7.12	5.21	17.89	6.39
120	0.62[a]	0.57	1.76[a]	0.40	5.00[a]	4.04	16.98	6.14
144	0.57[a]	0.57	1.57[a]	0.34	3.92[a]	3.40	14.50	6.43
168	0.75[a]	0.44	1.35[a]	0.41	2.94[a]	2.77	11.50	6.06

a n = 3

At the low dose, the mean half-lives in plasma were shorter in male rats (42.3 and 53.8 hours for [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole, respectively) than in female rats (129.0 and 117.3 hours for [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole, respectively) (Table 5). The difference was not as great at the high dose for either label (males, 61.7 and 55.3 hours; females, 64.7 and 79.7 hours). Absorption was considerably higher at the 10 mg/kg bw dose level in which 75.8 to 80.4% and 62.6 to 74.9% of the dose was measured for male and female rats, respectively, compared with 38.8 to 40.0% and 31.4 to 32.2% of the dose for male and female rats administered cyantraniliprole at 150 mg/kg bw, respectively (Table 6).

Both species of radiolabeled cyantraniliprole exhibited very similar kinetics (Table 5). Examination of the C_{max} and area-under-the-curve (AUC) values indicated a greater internal dose in female rats; this is consistent with greater $T_{1/2}$ in low dose females (10 mg/kg bw), whereas in high dose (150 mg/kg bw) groups, the $T_{1/2}$ in males and females were comparable. The AUC in female rats was approximately 2.5 fold greater than that in males at the same dose. The dose normalized AUC indicates that there was a decrease in the absorption for high doe (150 mg/kg bw) relative to the low dose (10 mg/kg bw). This is also reflected in the total percent dose absorbed (Table 6) derived from the bile duct cannulation study. Greater percentage of administered dose was absorbed with low dose administration. (≈ 76-80% of the administered dose for males and 63-75% for female) than the high dose (≈ 39-40% of the administered dose for males and 31-32% for females).

Table 5. Pharmacokinetic parameters in plasma following single oral dose administration of ^{14}C-cyantraniliprole

	10 mg/kg bw		150 mg/kg bw	
	Male	**Female**	**Male**	**Female**
[CN-^{14}C] cyantraniliprole				
$T_{1/2}$ (hr)	42.3±17.4	129.0±64.3	61.7±14.5	64.7±46.2
T_{max} (hr)	2.0±0.0	1.8±0.5	1.4±0.8	2.5±1.0
C_{max} (µg eq/g)	6.3±2.1	11.5±1.1	42.2±14.6	47.4±14.5
AUC (hr*µg/g)	194.5±126.7	609.0±194.1	1726.9±491.0	3591.7±2487.1
[PC-^{14}C] cyantraniliprole				
$T_{1/2}$ (hr)	53.8±19.8	117.3±20.1	55.3±9.2	79.7±28.4
T_{max} (hr)	2.5±1.0	1.6±0.8	1.0±0.7	1.3±11.3 [a]
C_{max} (µg eq/g)	4.8±0.6	10.4±0.8	42.2±9.9	52.2±13.3
AUC (hr*µg/g)	244.5±111.0	638.3±123.7	1834.2±1192.1	5474.1±2300.2

[a] Mean ± SD for n = 4 rats/sex

[b] One animal excluded from calculation

Data excerpted from page 45 of the report.

Table 6. Percent dose absorbed based on biliary elimination and material balance following single oral dose administration of ^{14}C-cyantraniliprole (48 hours after dosing).

Sample	10 mg/kg bw		150 mg/kg bw	
	Male	**Female**	**Male**	**Female**
[CN-^{14}C] cyantraniliprole				
Urine	42.25±7.69	33.03±4.66	20.74±2.59	16.05±1.61
Faeces	17.49±3.82	21.58±5.08	54.90±8.48	59.62±6.88
Bile	27.68±14.67	15.71±3.36	16.04±4.35	10.00±1.12
Cage Wash	3.16±1.55	3.00±0.59	2.20±0.83	5.67±4.62
Carcass[b]	5.66±1.68	13.09±4.15	2.94±0.94	5.10±1.49
G.I. track	0.20±0.05	0.75±0.37	0.23±0.16	0.25±0.15
G.I. contents	0.57±0.20	1.79±0.27	1.95±0.71	1.67±0.90
Total	97.01±8.95	88.95±3.37	99.00±1.31	98.35±2.70
Absorbed dose[c]	**75.80±11.44**	**62.58±6.23**	**39.96±7.22**	**31.39±3.40**
[PC-^{14}C] cyantraniliprole				
Urine	38.92±9.82	35.51±18.40	22.50±7.57	14.14±10.01
Faeces	13.48±2.23	20.04±8.80	39.29±22.18	38.25±27.02
Bile	36.54±7.90	27.20±7.13	11.55±0.96	11.34±1.86
Cage Wash	1.83±0.95	2.62±1.03	3.31±0.70	1.50±0.30
Carcass	4.81±0.73	11.53±1.17	3.55±3.04	4.68±2.68
G.I. track	0.15±0.05	0.69±0.25	1.22±2.30	2.01±3.28
G.I. contents	0.69±0.43	3.99±3.84	14.41±27.96	26.64±32.43
Total	96.43±3.18	101.58±22.55	95.82±8.14	98.56±3.77
Absorbed dose	**80.43±4.64**	**74.93±18.46**	**38.81±6.25**	**32.17±7.88**

[a] Mean and SD for n= 4 rats per sex

[b] Includes whole blood collected at time of sacrifice

[c] Sum of urine + bile + carcass including GI tract tissues, but not contents

Data excerpted from pages 73-74 of the study report.

Distribution

The tissue distribution data showed no appreciable difference between dosing with the [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole in terms of tissue distribution. The majority of the dose was initially associated with the GI tract contents and subsequently showed uptake and distribution to all tissues. The percent recovery and tissue concentration data showed that female rats retained a greater proportion of ^{14}C residues than male rats. The general patterns of decline of the plasma and tissue ^{14}C residue concentrations at high and low doses were generally similar (Figure 1). These observations were consistent with the shorter elimination half-life in male rats compared with female rats. After 168 hours following dosing, skin, liver, and muscle generally have slightly higher residue level than other tissues examined, but the level expressed as percentage of the administered dose was less than 1% (Tables 7 & 8). As indicated before, the tissue distribution data showed no appreciable difference between dosing with the [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole in terms of tissue distribution. Hence, the tissue concentrations (µg equiv/g) at T_{max} following [CN-^{14}C]-cyantraniliprole could be considered as representative data for [PC-^{14}C]-cyantraniliprole, and the data were summarized in Table 7a for selective tissues some of which showed toxicological effect.

Figure 1.Concentration (µg equiv/g) in plasma following a 10 or 150 mg/kg bw single oral dose of [CN-^{14}C] cyantraniliprole (Figure excerpted from page 88 of the report).

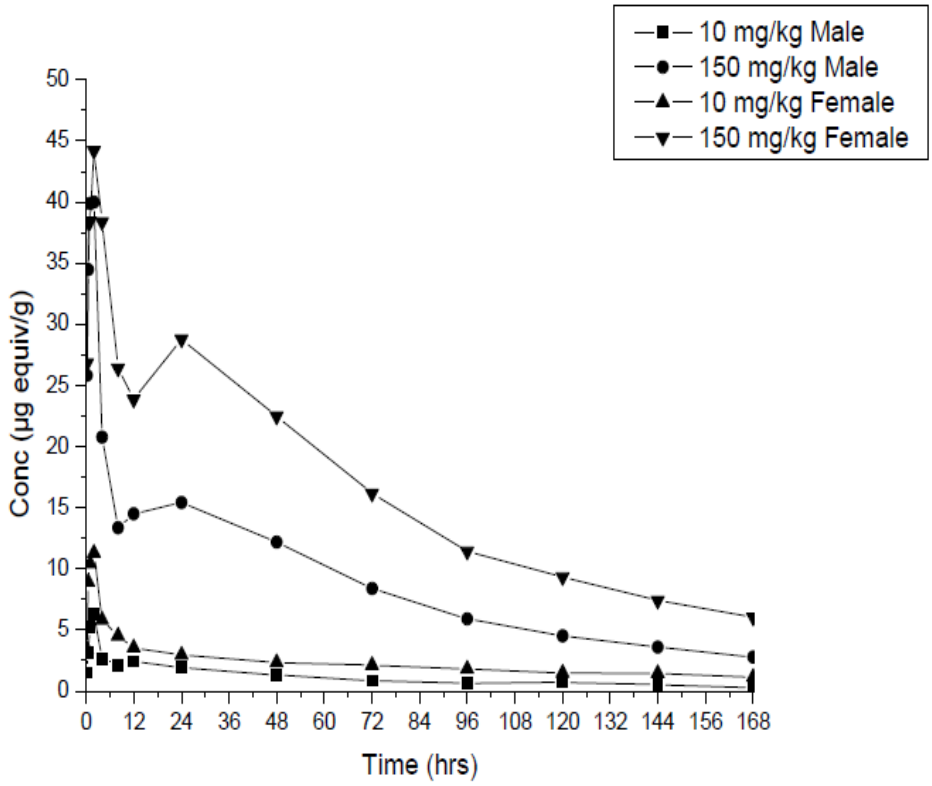


Table 7. Percent of dose recovered at 168 hours in tissues in male and female rats following oral gavage with 10 mg/kg radiolabeled cyantraniliprole

Tissue	[CN-^{14}C]-cyantraniliprole				[PC-^{14}C]-cyantraniliprole			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Skin[a]	**0.3846**	**0.3385**	**0.9016**	**0.2831**	**0.4243**	**0.1478**	**1.1240**	**0.3427**
Whole blood	0.0561	0.0192	0.2649	0.0979	0.1055	0.0174	0.2564	0.2067
Bone marrow	0.0002[b]	NA	0.0009	0.0002	0.0004	0.0002	0.0008	0.0007
Brain	0.0010[c]	NA	0.0052	0.0016	0.0009	0.0001	0.0062	0.0023
Fat[a]	0.0209	0.0102	0.9258	0.4620	0.0255	0.0096	1.1834	0.3353
RBC (terminal)	0.0050	0.0032	0.0183	0.0053	0.0063	0.0023	0.0209	0.0069
Heart	0.0057	0.0031	0.0256	0.0036	0.0078	0.0018	0.0246	0.0136
Lungs	0.0098	0.0045	0.0394	0.0077	0.0130	0.0015	0.0399	0.0151
Spleen	0.0015	0.0005	0.0065	0.0026	0.0018	0.0005	0.0052	0.0022
Liver	**0.1375**	**0.0373**	**0.3612**	**0.0465**	**0.1760**	**0.0259**	**0.3970**	**0.1131**
Kidney	0.0158	0.0050	0.0395	0.0082	0.0179	0.0027	0.0416	0.0152
G.I. tract	0.0390	0.0282	0.1664	0.0643	0.0284	0.0076	0.1147	0.0249
G.I. contents	**0.1826**	**0.0273**	**0.3751**	**0.0625**	**0.1446**	**0.0328**	**0.4573**	**0.0237**
Pituitary	<LOQ	NA	0.0015	0.0015	0.0000	NA	0.0008[c]	0.0007
Thyroid	<LOQ	NA	0.0009	0.0007	0.0002[b]	NA	0.0013[c]	0.0006
Thymus	0.0018	0.0011	0.0061	0.0008	0.0015	0.0004	0.0073	0.0017
Ovaries	—	—	0.0072	0.0018	—	—	0.0050	0.0013
Testes	0.0090	0.0045	—	—	0.0135	0.0021	—	—
Pancreas	0.0028	0.0011	0.0199	0.0012	0.0026	0.0015	0.0341	0.0276
Adrenals	0.0022	0.0007	0.0094	0.0030	0.0017	0.0003	0.0102[a]	0.0032
Plasma (terminal)	0.0149	0.0073	0.0857	0.0322	0.0193	0.0064	0.0849	0.0224
Uterus	—	—	0.0188	0.0099	—	—	0.0101	0.0038
Muscle[a]	**0.1270**	**0.0672**	**0.5900**	**0.2004**	**0.2136**	**0.0351**	**0.6249**	**0.1793**
Bladder	0.0008	0.0006	0.0035	0.0013	0.0006	0.0001	0.0040	0.0026
Bone[a]	0.0265	0.0122	0.1003	0.0312	0.0342	0.0071	0.0946	0.0383

[a] Percentages for tissues collected as partial samples were adjusted to estimate recovery in the full tissue weight as percent of pre-sacrifice body weight: skin (19%), fat (7%), muscle (40%), and bone (7.3%).

[b] n = 2 [c] n = 3

Data excerpted from the following pages of the report: 197 & 199 for [CN-^{14}C]; 212 & 214 for [PC-^{14}C].

Table 7a. Representative tissue concentrations (µg/equiv/g) at T_{max} following single oral dosing with [CN-^{14}C]-cyantraniliprole .

Tissue	10 mg/kg		150 mg/kg	
	Males (T_{max}=2.0h)	Females (T_{max}=1.8h)	Males (T_{max}=1.4h)	Females (T_{max}=2.5h)
Adrenal	16.78±3.84	19.21±5.10	49.67±12.90	126.78±23.98
Brain	0.53±0.11	0.65±0.22	2.40±0.36	3.73±0.85
Fat	9.63±3.36	12.33±3.29	40.18±7.24	76.23±21.33
G.I . tract	28.86±4.33	28.22±3.56	1198.60±1026.30	408.53±365.19
G.I. content	48.25±9.71	40.23±4.12	2405.10±962.35	1783.50±589.99
Kidney	9.86±1.61	8.70±2.54	41.03±1.77	49.63±13.09
Liver	54.26±5.94	54.37±8.23	153.62±16.46	170.72±25.91
Plasma	10.16±1.34	10.83±4.14	39.53±4.51	51.16±14.37
Skin	4.69±0.89	6.01±2.26	21.44±1.46	36.89±6.57
Thyroid	17.96±8.38	26.81±20.65	87.25±54.58	136.11±62.01

Data excerpted from pages 57, 58, 61, & 62 of the report.

Table 8. Percent of dose recovered at 168 hours in tissues in male and female rats following oral gavage with 150 mg/kg radiolabeled cyantraniliprole								
	[CN-^{14}C]-cyantraniliprole				[PC-^{14}C]-cyantraniliprole			
	Male		Female		Male		Female	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Skin[a]	**0.2477**	**0.0815**	**0.5177**	**0.2552**	**0.0670**	**0.0328**	**0.6326**	**0.1110**
Whole blood	0.0433	0.0180	0.1973	0.1229	0.0291	0.0148	0.2413	0.1057
Bone marrow	0.0002	0.0000	0.0008[c]	0.0002	0.0002[d]	NA	0.0004	0.0002
Brain	<LOQ	NA	0.0028[c]	0.0002	<LOQ	NA	0.0030	0.0008
Fat[a]	0.0135	0.0015	0.1959	0.0276	0.0092[c]	0.0027	0.2938	0.1319
RBC (terminal)	0.0025	0.0003	0.0528	0.0495	0.0021	0.0005	0.0152[c]	0.0039
Heart	0.0037	0.0012	0.0090	0.0052	0.0016	0.0007	0.0133	0.0049
Lungs	0.0053	0.0014	0.0183	0.0101	0.0028	0.0011	0.0283	0.0100
Spleen	0.0008	0.0002	0.0028	0.0014	0.0005[c]	0.0001	0.0033	0.0007
Liver	0.0777	0.0083	0.2006	0.0793	0.0431	0.0086	0.2387	0.0439
Kidney	0.0070	0.0009	0.0233	0.0113	0.0037	0.0013	0.0249	0.0058
G.I. tract	0.0164	0.0019	0.0505	0.0211	0.0087	0.0036	0.0782	0.0209
G.I. contents	**0.0902**	**0.0137**	**0.2950**	**0.0891**	**0.0456**	**0.0229**	**0.1827**	**0.0606**
Pituitary	<LOQ	NA	0.0003[c]	0.0002	<LOQ	NA	0.0003[c]	0.0001
Thyroid	<LOQ	NA	0.0007[c]	0.0004	<LOQ	NA	0.0009	0.0005
Thymus	0.0010	0.0002	0.0044	0.0022	0.0006	0.0003	0.0050	0.0014
Ovaries	—	—	0.0019[c]	0.0002	—	—	0.0035	0.0010
Testes	0.0051	0.0011	—	—	0.0027	0.0012	—	—
Pancreas	0.0015	0.0006	0.0051	0.0025	0.0007	0.0002	0.0111	0.0051
Adrenals	0.0010	0.0002	0.0020	0.0006	0.0007	0.0005	0.0047	0.0026
Plasma (terminal)	0.0108	0.0032	0.0523	0.0327	0.0056	0.0032	0.0611	0.0105
Uterus	—	—	0.0046	0.0025	—	—	0.0101	0.0037
Muscle[a]	**0.0775**	**0.0157**	**0.2659**	**0.1423**	**0.0479**	**0.0121**	**0.3467**	**0.1101**
Bladder	0.004[b]	NA	0.0016[c]	0.0002	<LOQ	NA	0.0023	0.0010
Bone[a]	0.0212	0.0076	0.0595	0.0290	0.0117[c]	0.0053	0.0744	0.0145

[a] Percentages for tissues collected as partial samples were adjusted to estimate recovery in the full tissue weight as percent of pre-sacrifice body weight: skin (19%), fat (7%), muscle (40%), and bone (7.3%).

[b] n = 2

[c] n = 3

[d] n = 1

Data excerpted from the following pages of the report: 198 & 200 for [CN-^{14}C]; 213 & 215 for [PC-^{14}C].

Excretion

The elimination patterns showed was no appreciable difference between rats administered [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole (Table 9). Greater percentage of the administered dose was eliminated via faecal route (≈ 47 -80%) relative to the urinary elimination (≈12-35%). Rats given a single 10 mg/kg bw dose of either species of cyantraniliprole excreted a greater percentage of the dose in urine (≈22 to 35%) than rats dosed with 150 mg/kg bw (≈12% to 15%). The percent recovery in rats at the 10 mg/kg dose level was 22.0 to 34.6% for urine, 46.8–61.6% for faeces, and 1.1 to 5.3% for tissues. At 150 mg/kg level, the percent recovery was 11.8 to 14.4% in urine, 77.6 to 80.1% in feces, and 0.25 to 2.45% in the tissues. For both dose levels and labels, the majority of the dose was excreted by 24–48 hours after administration; only a representative data set on [CN-^{14}C]-

labelled animals are presented (Tables 10 & 11) as there was no appreciable difference observed in the elimination profile between [CN-^{14}C]-labelled and [PC-^{14}C]- labelled animals.

Table 9. Route of excretion and total recovery of radiolabelled cyantraniliprole in rat

Radiolabel cyantraniliprole	Target dose (mg/kg bw)	Sex	% of dose				
			Urine	Faeces	Cage wash	Tissues[a]	Total recovery[b]
[CN-^{14}C]	10 mg/kg bw	M	27.68	61.53	5.62	1.14	96.54
		F	22.03	61.59	5.35	4.25	92.64
	150 mg/kg bw	M	14.77	77.57	1.66	0.68	94.96
		F	13.17	78.60	1.12	2.45	95.06
[PC-^{14}C]	10 mg/kg bw	M	34.56	46.75	5.23	1.67	88.25
		F	23.65	60.64	3.40	5.35	93.00
	150 mg/kg bw	M	11.77	80.10	2.27	0.25	94.54
		F	12.93	77.57	1.08	2.30	93.72

[a] Percentage for tissue was experimentally measured amount in carcass and all tissues except plasma and red blood cells.

[b] A separate group was dosed to determined if any radioactivity was excreted as $^{14}CO_2$. All samples were below LOD.

Data excerpted from page 46 of the report.

Table 10. Percent of dose eliminated in urine following a 10 or 150 mg/kg bw single dose of [CN-^{14}C]-cyantraniliprole (Table excerpted from page 47 of the report)

Collection Time (hours)	Percent of Dose							
	10 mg/kg bw				150 mg/kg bw			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6	14.13	3.00	4.44	2.06	6.76	0.74	2.36	0.64
12	7.67	3.32	6.84[a]	1.92	4.23	0.75	2.76	0.72
24	2.88	1.07	4.57	0.44	1.86	0.29	3.52	0.82
48	1.45	0.37	4.47	3.23	1.01	0.39	3.15	1.15
72	0.54	0.15	1.34	1.24	0.38	0.13	0.59	0.23
96	0.32	0.16	0.77	0.28	0.20	0.05	0.36	0.08
120	0.22	0.15	0.39	0.12	0.15	0.07	0.21	0.04
144	0.23	0.06	0.18	0.12	0.09	0.02	0.15	0.02
168	0.22	0.07	0.17	0.06	0.07	0.03	0.07	0.02

a n = 3

Table 11. Percent of dose eliminated in feces following a 10 or 150 mg/kg bw single dose of [CN-^{14}C]-cyantraniliprole (Table excerpted from page 48 of the study report)

Collection Time (hours)	Percent of Dose							
	10 mg/kg bw				150 mg/kg bw			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6	0.35[a]	0.27	0.50[b]	NA	0.15[b]	NA	0.28	0.34
12	19.28	6.09	12.74	8.04	6.23	3.87	5.53	3.98
24	26.94	5.20	28.62	7.00	47.81	11.22	21.05	13.78
48	10.52	3.99	11.26	4.44	19.54	9.68	42.57	12.46
72	1.95	0.27	3.49	0.40	2.65	2.06	5.51	2.24
96	1.02	0.50	1.94	0.25	0.50	0.13	1.68	0.50
120	0.71	0.25	1.52	0.46	0.34	0.05	0.86	0.25
144	0.46	0.12	1.16	0.11	0.30	0.12	0.54	0.13
168	0.38	0.01	0.61	0.09	0.14	0.05	0.59	0.44

a n = 3
b n = 2

Metabolism

Cyantraniliprole and its metabolites were extracted from pooled urine and cage wash samples and from pooled faeces and bile and identified using HPLC with confirmation using LC/MS. In urine, the metabolites in common for both sexes and both the [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole doses that were observed by mass spectral analysis were bis-hydroxy-HGW86, IN-N7B69, IN-MYX98, IN-HGW87, IN-NBC94, IN-MLA84, and IN-J9Z38 as well as parent cyantraniliprole (Tables 12 &13). IN-DCB80 was observed in urine from the [PC-^{14}C]-cyantraniliprole dose group. The identified components as the percent of dose showed parent cyantraniliprole present as approximately 5% in the 10 mg/kg bw dose females (both labels) but only ≤1% of the dose in the 10 mg/kg bw males. At the high dose, cyantraniliprole accounted for <2% of the dose at all dose levels, labels, and both sexes. IN-MYX98 and IN-N7B69 both appear to be the major metabolites in urine, regardless of sex, label, or dose level followed by bis-hydroxy-cyantraniliprole. IN-MYX98 ranged from 4–11% of the dose, IN-N7B69 ranged from 0.6 to 4%, and bis-hydroxy-cyantraniliprole ranged from undetected to 3% of the dose. Unidentified peaks accounted for approximately 5% or less for all samples except for the 10 mg/kg [CN-^{14}C]-cyantraniliprole urine sample in which the unidentified peaks consisted of approximately 15% of the dose. In all cases, the unknown fraction was comprised of a mixture of components, none of which exceed 5% of the administered dose. The profiles of metabolites at the low and high (10 and 150 mg/kg bw) dose levels were qualitatively similar. There may be a slight sex difference in the relative amounts of IN-MYX98 and IN-N7B69 with females tending to produce more IN-MYX98 than IN-N7B69 but males produced similar amounts of each metabolite. This was true for both labels and both dose levels.

In faeces, the metabolites in common for both sexes and both the [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole doses that were observed by mass spectral analysis were bis-hydroxy-cyantraniliprole (Table 14 &15), IN-N7B69-O-glucoronide, IN-DCB80, IN-N7B69, IN-MYX98, IN-HGW87, cyantraniliprole, IN-NBC94, IN-MLA84, and IN-

J9Z38, as well as components m/z 471, m/z 457, and m/z 489. The identified components as the percent of dose showed that parent cyantraniliprole present as approximately 15–16% in the 10 mg/kg bw dose females (both labels), but only about 5% of the dose in the 10 mg/kg bw males. At the high dose, cyantraniliprole accounted for 55–66% of the dose and at the low dose, it accounted for 5-16% of the dose. IN-MYX98 was the major metabolites in faeces, regardless of sex, label, or dose level, ranging from 4–14% of the dose. Other significant metabolites included bis-hydroxy-cyantraniliprole, IN-DBC80, IN-NBC94, IN-N7B59, IN-HGW87, IN-MLA84, and IN-J9Z38. Unidentified peaks accounted for approximately 7% or less for all samples except for the 10 mg/kg [CN-^{14}C]-cyantraniliprole urine sample in which the unidentified peaks consisted of approximately 17% of the dose. In all cases, the unknown fraction was comprised of a mixture of components, none of which exceed 5% of the administered dose. The profiles of metabolites at the 10 mg/kg bw doses were similar to each other when compared by radiolabel and sex. This was also true at the 150 mg/kg bw dose levels.

In bile, the metabolites in common for both sexes and both the [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole doses that were observed by mass spectral analysis were very diverse and included glucuronides of IN-N7B69, IN-MLA84, and IN-NBC94; IN-MLA84; and IN-J9Z38 (Tables 16 & 17). The bile was very rich in metabolites at both dose levels, both labels, and in both sexes. No single metabolite was greater than 5% of the dose. Unidentified peaks accounted for approximately 6% or less for all samples except for the male rats receiving the 10 mg/kg dose rate in which the unidentified peaks consisted of approximately 12% of the dose. In all cases, the unknown fraction was comprised of a mixture of components, none of which exceed 5% of the administered dose.

The metabolic pathway in rats can be summarized as follows (Figure 2): Cyantraniliprole is readily hydroxylated to form IN-N7B69 and IN-MYX98. IN-N7B69 is further metabolized to a glucuronide. Cyantraniliprole undergoes ring closure to generate IN-J9Z38 which is then in turn hydroxylated to form IN-NBC94, its carboxylic acid, and its glucuronide conjugate. IN-MYX98 is also metabolized to the closed-ring metabolite IN-MLA84, which like IN-NBC94, is further oxidized to a hydroxyl, a carboxylic acid, and the glucuronide of the hydroxyl metabolite. Further, the hydroxylated metabolite IN-MYX98 can be N-dealkylated to form IN-HGW87 as well as being hydroxylated a second time to form the bis-hydroxy-cyantraniliprole. Cyantraniliprole can also be hydroxylated on the pyridine ring, followed by a ring closure analogous to the conversion of cyantraniliprole to IN-J9Z38. Cyantraniliprole can also be N-dealkylated at the carbonyl bridge to form IN-DBC80.

Table 12. Summary of [CN-^{14}C]cyantraniliprole and metabolites in urine as percent of administered dose

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
BisHOHGW86	1.40	ND	0.45	0.31
IN-N7B69	4.43	0.35	4.53	0.65
IN-MYX98	4.52	11.47	4.34	4.88
IN-HGW87	ND	0.54	0.13	0.67
DPX-HGW86	0.33	5.42	1.37	1.83
IN-NBC94	ND	ND	0.42	0.25
Isomer of IN-MYX98	0.86	0.03	0.70	0.27
IN-MLA84	ND	0.16	0.09	0.46
IN-J9Z38	ND	ND	ND	0.20
Unknown	14.55	3.73	0.82	2.28
Total	26.09	21.69	12.85	11.80

Data excerpted from page 78 of the study report.

Table 13. Summary of [PC-^{14}C]cyantraniliprole and metabolites in urine as percent of administered dose

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
BisHOHGW86	3.04	0.50	1.08	0.47
IN-DBC80	2.10	0.91	0.43	0.49
IN-N7B69	13.62	1.74	3.97	1.21
m/z 633 Gluc	0.56	ND	0.45	ND
IN-MYX98	4.07	8.55	2.10	3.95
IN-HGW87	0.23	0.67	0.18	0.24
DPX-HGW86	1.09	3.58	0.77	1.35
IN-NBC94	0.60	0.00	0.36	0.07
Isomer of IN-MYX98	1.40	0.50	0.52	0.12
IN-MLA84	0.27	0.32	0.14	1.28
IN-J9Z38	0.04	0.24	0.02	0.39
Unknown	5.38	4.66	1.03	2.69
Total	32.40	21.68	11.05	12.26

Data excerpted from page 78 of the report.

Table 14. Summary of [CN-^{14}C]DPX-HGW86 and metabolites in feces as percent of administered dose

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
m/z 655	3.11	ND	ND	ND
BisHOHGW86	**8.12**	**2.04**	**2.45**	**1.04**
N7B69-O-Gluc m/z 665	ND	0.11	ND	ND
IN-N7B69	4.91	2.40	1.96	0.61
IN-MYX98	**10.49**	**14.42**	**5.46**	**6.73**
IN-HGW87	2.14	4.10	1.14	3.05
DPX-HGW86	**5.06**	**16.77**	**55.78**	**55.01**
IN-NBC94	2.41	3.03	0.88	0.36
Isomer of IN-MYX98	2.10	0.49	1.51	1.29
IN-MLA84	1.19	3.36	0.69	2.17
IN-J9Z38	0.30	2.79	0.48	0.92
m/z 471	ND	ND	ND	0.38
Unknown	17.25	7.11	3.23	3.38
Total	57.08	56.61	73.58	74.94

Data excerpted from page 81 of the report.

Table 15. Summary of PC-^{14}C]cyantraniliprole and metabolites in feces as percent of administered dose

Identified component	Metabolite as Percent of Dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
N7B69-O-Gluc m/z 665	ND	ND	ND	0.73
BisHOHGW86	**5.59**	**1.93**	**1.64**	**0.50**
IN-DBC80	**5.30**	2.56	0.66	ND
IN-N7B69	3.58	1.96	0.17	0.31
IN-MYX98	**9.25**	**17.18**	**3.59**	**6.37**
IN-HGW87	1.46	5.52	1.28	2.26
DPX-HGW86	**5.38**	**15.04**	**65.59**	**59.37**
IN-NBC94	2.57	2.94	0.45	1.08
Isomer of IN-MYX98	3.20	0.33	1.20	ND
IN-MLA84	0.76	2.93	0.73	2.18
IN-J9Z38	0.19	2.83	0.08	ND
m/z 457	0.10	ND	ND	ND
m/z 471	0.05	ND	ND	ND
Unknown	6.94	3.48	3.01	1.81
Total	44.37	56.71	78.40	74.62

Data excerpted from page 81 of the report.

Table 16. Summary of [CN-^{14}C]cyantraniliprole and metabolites in bile as percent of administered dose

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
HO-MLA84-O-Gluc m/z 633	2.15	0.47	1.57	0.69
N7B69-O-Gluc m/z 665	**4.00**	**2.93**	**1.27**	**1.67**
NBC94-O-Gluc m/z 647	**4.78**	**4.83**	**3.58**	**2.18**
m/z 592	ND	ND	0.80	ND
m/z 442	2.11	0.81	ND	ND
m/z 631-1 Gluc	ND	0.23	ND	ND
m/z 592 & 442	ND	ND	ND	0.86
m/z 574	ND	ND	0.24	ND
m/z 574 & 631	ND	ND	0.35	ND
m/z 485	ND	ND	0.34	ND
m/z 634 Gluc	ND	1.01	1.18	1.35
m/z 647-2 Gluc	**2.14**	0.49	0.23	0.20
m/z 647-3 Gluc	0.61	ND	ND	ND
m/z 631-2 Gluc	ND	ND	ND	0.47
IN-MLA84	ND	ND	0.16	ND
Unknown	11.91	4.94	6.32	2.58
Total	27.70	15.71	16.04	10.00

Data excerpted from page 84 of the report.

Table 1. Summary of [PC-^{14}C]cyantraniliprole and metabolites in bile as percent of administered dose

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
HO-MLA84-O-Gluc m/z 633	0.97	2.22	1.15	0.79
N7B69-O-Gluc m/z 665	2.78	3.60	1.07	1.93
NBC94-O-Gluc m/z 647	2.62	3.73	2.25	2.08
m/z 592	ND	ND	1.19	ND
m/z 442	2.20	1.98	ND	0.39
m/z 574 & 631	ND	ND	0.15	ND
m/z 647-2 Gluc	4.35	1.67	0.22	1.07
m/z 649 Gluc	ND	ND	ND	0.20
m/z 473	ND	ND	ND	0.24
m/z 647-3 Gluc	3.60	1.57	0.96	ND
m/z 631 Gluc	ND	0.29	ND	0.08
IN-NBC94	3.41	0.66	0.17	1.21
m/z 344	2.27	2.21	0.09	0.04
m/z 344 & 485	ND	ND	0.55	ND
m/z 617 Gluc	ND	ND	ND	0.75
m/z 617-2 Gluc	ND	ND	ND	0.15
m/z 485	ND	ND	0.21	ND
m/z 471-2	1.25	1.12	ND	ND
m/z 471-3	ND	0.45	ND	ND

Identified component	Metabolite as percent of dose			
	10 mg/kg bw		150 mg/kg bw	
	Male	Female	Male	Female
IN-MLA84	ND	1.55	0.97	0.70
IN-J9Z38	0.47	0.61	0.06	0.07
Unknown	12.52	5.54	2.51	1.64
Total	36.44	27.20	11.55	11.34

Data excerpted from page 85 of the report.

The proposed pathway for the metabolism of cyantraniliprole in rats is shown in Figure 2, page 21 of the data evaluation repor.

III. CONCLUSION

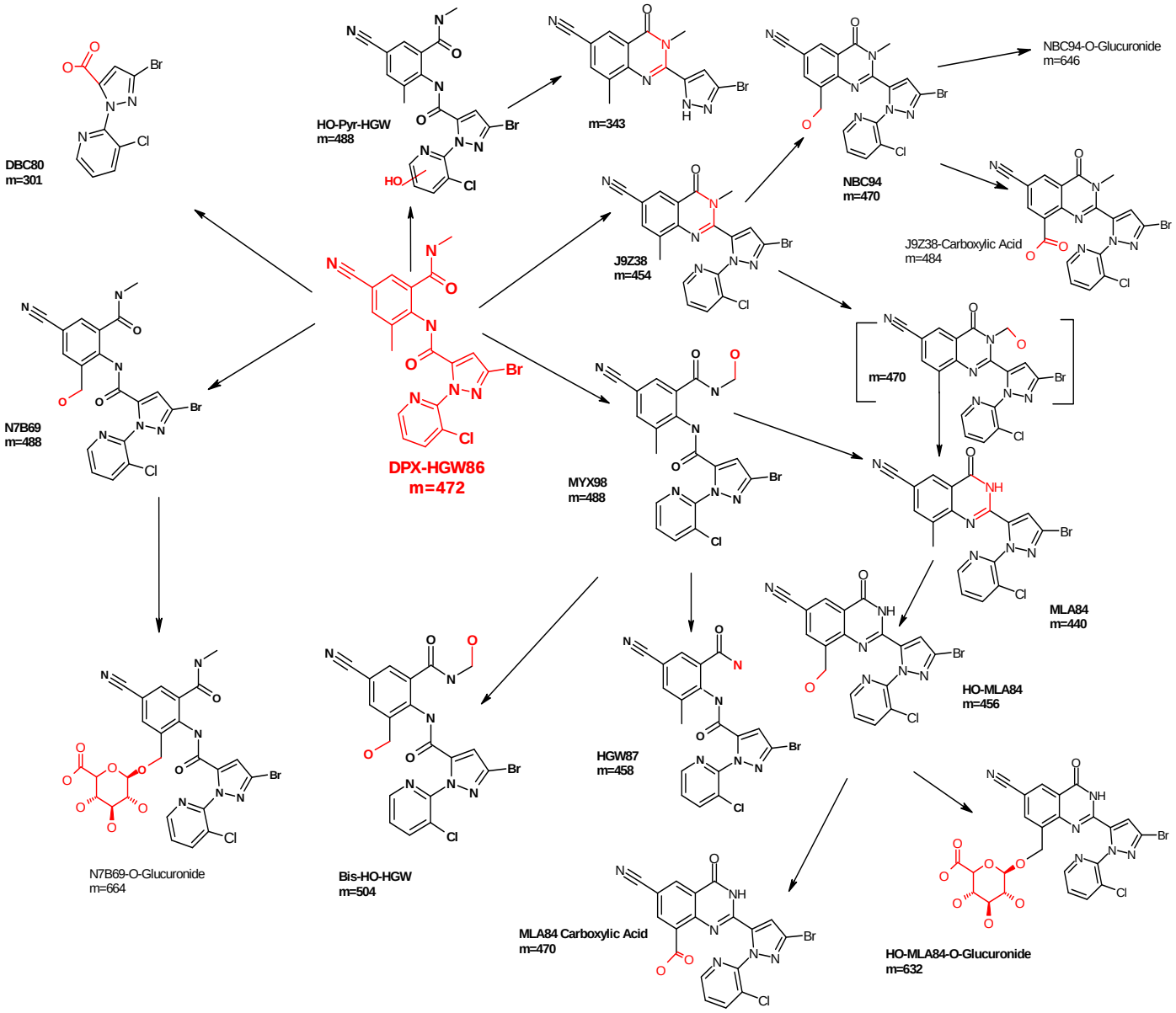
The data indicated that with oral administration of cyantraniliprole at low (10 mg/kg bw) and high (150 mg/kg bw) doses, it was absorbed readily. The majority of the absorption occurred during the first 48 hours and the peak plasma concentration was reached at approximately 2 hour after dosing irrespective of the position of label, sex of the test animal, and dose level. How ever, with 10 mg/kg bw, the $T_{1/2}$ was lower in male relative to females. The values of AUC was also substantially smaller in male than in females. With dose-normalized AUC, the data suggested a decrease in absorption at high dose in comparison to the low dose.

The majority of the dose was initially associated with the GI tract contents and subsequently showed uptake and distribution to all tissues. The percent recovery and tissue concentration data showed that female rats retained a greater proportion of ^{14}C residues than male rats. After 168 hours following dosing, skin, liver, and muscle generally have slightly higher residue level than other tissues examined, but the level expressed as percentage of the administered dose was less than 1%.

The metabolism results suggested that Cyantraniliprole was hyrooxylated to form IN-N7B69 and IN-MYX98. IN-N7B69 was further metabolized to a glucuronide. Cyantraniliprole could also undergo ring closure to generate IN-J9Z38 which was hydroxylated to form IN-NBC94, its carboxylic acid, and its glucuronide conjugate. IN-MYX98 was also metabolized to the closed-ring metabolite IN-MLA84, which, like IN-NBC94, was further oxidized to a hydroxylated metabolite, a carboxylic acid, and the glucuronide of the hydroxyl metabolite. Further, the hydroxylated metabolite IN-MYX98 could be N-dealkylated to form IN-HGW87 as well as being hydroxylated a second time to form bis-hydroxy-HGW86. Cyantraniliprole can also be hydroxylated on the pyridine ring, followed by a ring closure analogous to the conversion of cyantraniliprole to IN-J9Z38. Cyantraniliprole could also be N-dealkylated and cleaved at the carbonyl bridge to form IN-DBC80.The metabolites which were found to be greater than 5% of the administered dose were bis-hyroxy-cyantraniliprole, IN-N7B69, IN-MYX98, INDBC80, and the parent compound.

There was essentially no difference in elimination between rats administered [CN-^{14}C]-cyantraniliprole or [PC-^{14}C]-cyantraniliprole. Rats given a single 10 mg/kg bw dose of either species of cyantraniliprole excreted a greater percentage of the dose in urine (22.0 to 34.6%) than rats dosed with 150 mg/kg bw (11.8 to 14.8%). For both dose levels and labels, the majority of the dose was excreted by 24 to 48 hours after administration. The percent recovery in rats at the 10 mg/kg dose level was 22.0 to 34.6% for urine, 46.8 to 61.6% for faeces, and 1.1 to 5.3% for tissues. When rats were given 150 mg/kg bw, greater percentage of the dose excreted in the faeces (77.6 to 80.1%). For all groups in which material balance was measured, the mean percentage for total recovery by 7 days after dosing ranged from 88.3 to 96.5%. The data also indicate that there was no appreciable tendency for bioaccumulation to occur for cyantraniliprole.

Figure 2. Proposed metabolic pathway of cyantraniliprole in the rat: Single dose study
(Figure excerpted from page of 171 of the study)



Global Primary Reviewer: Whang Phang, PhD

IIA 5.7.1/01

Report: Malley, L.A. (2006); DPX-HGW86 technical: Acute oral neurotoxicity study in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: duPont-16996. April 19, 2006. MRID 48119950. Unpublished.

Guidelines: OPPTS 870.6200 (1998)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In an acute neurotoxicity study (MRID 48119950), male and female Crl:CD®(SD)IGS BR rats (12 rats/sex/dose) received cyantraniliprole (93.4%; HGW86-141) in a single gavage dose in polyethylene glycol (PEG 400) at a volume of 4 mL/kg bw. Doses were 0, 250, 1000, or 2000 mg/kg bw. A neurobehavioral test battery, consisting of motor activity and functional observational battery (FOB) assessments, was conducted on all study rats prior to dosing, approximately 2 hours after dosing (Day 1) and on Days 8 and 15. Other parameters evaluated included body weight, body weight gain, and clinical signs. On test day 17, 6 rats/sex/dose were perfused in situ with fixative. Neuropathological examinations of the peripheral and central nervous systems and selected muscle tissues from control and high dose rats were carried out.

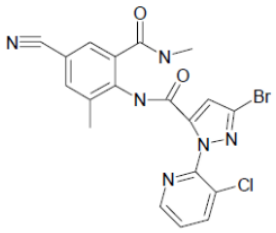
Under the conditions of this study, no test substance-related changes were found in any treatment groups for the following evaluations: body weight, body weight gain, food consumption, food efficiency, mortality, clinical observations, forelimb or hindlimb grip strength, hind limb foot splay, body temperature, rearing, duration or number of movements, or any of the other behavioral parameters in the FOB. In addition, there were no gross or microscopic test substance-related morphological changes in the nervous system tissues.

The NOAEL for this acute neurotoxicity was 2000 mg/kg bw (highest dose tested) for male and female rats. No LOAEL was established based on the absence of toxicity at any dosed groups in this study.

This study is fully reliable (acceptable/guideline) and satisfies the data requirements for an acute neurotoxicity study (OPPTS 870.6200).

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole technical (DPX-HGW86) 1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]
		
	Lot/Batch #:	HGW86-141
	Purity:	93.4%
	Description:	Off-white solid
	CAS #:	736994-63-1
	Stability of test compound:	The test substance was mixed uniformly, was at the target levels and stable for 5 hours at room temperature.
2.	Vehicle and/or positive control:	Polyethylene glycol (PEG 400)
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)IGS BR
	Age at dosing:	Approximately 42 days old
	Weight at dosing:	174.6–231.7 g for males; 129.4–182.1 g for females
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	14 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4.	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not reported
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   30-August-2005 to 19-April-2006

2. Animal assignment and treatment

   The reported stated that the dose levels were selected based on the results of a range-finding study, in which no test substance-related effects were observed on body weight, clinical signs of toxicity, grip strength, foot splay or motor activity at dosages of 0, 250, 1000, and 2000 mg/kg in 5 males or 5 females. For the main study, doses of 0, 250, 1000, and 2000 mg/kg bw were selected. The test rats (12/sex/dose) received a single dose of cyantraniliprole by gavage (Table 1). The test substance was administered in polyethylene glycol (PEG 400) at a volume of 4 mL/kg bw.

   Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received the vehicle. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study Design

No./ Sex/Dose	Dose (mg/kg bw)[a]
12	0
12	250
12	1000
12	2000

[a]: Test substance was administered in PEG 400 at a volume of 4 mL/kg.

3. Dosing formulation and analysis

   The test materials were prepared on the day of dosing. The stability, homogeneity, and concentration of cyantraniliprole in the dosing preparations were checked by analysis using HPLC. The test substance was at the targeted dose levels (98.1-100%), was homogeneous throughout the preparations, and stable for at least 5 hours at room temperature. Based on this information, it can be concluded that the animals received the targeted dose levels during the study. Dose formulations were prepared with a correction for the purity of the test substance.

4. Statistics: Statistical methods used in this study are presented in Table 2.

Table 2. Statistical Methods

Parameter	Preliminary test	Method of statistical analysis

		If preliminary test is not significant	If preliminary test is significant
Body weight Body weight Gain Food consumption	Levene's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Motor activity[b] Grip strength Foot splay Body temperature Rearing	Levene's test for homogeneity and Shapiro-Wilk test for normality[d]	Repeated measures analysis of variance followed by linear contrasts	Sequential application of the Jonckheere-Terpstra trend test[c]
Incidence of FOB Descriptive parameters	None	Cochran-Armitage test for trend[e]	

[a] If the Shapiro-Wilk test is not significant but Levene's test is significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test is followed by Dunn's test.

[b] Test day and 10-minute interval were used as repeated-measure factors.

[c] If there is a significant lack of monotonicity (*i.e.*, Linear Trend:Dose is not significant, and Quadratic Trend:Dose is significant), Dunn's test was used instead of Jonckheere-Terpstra.

[d] Normalizing or variance stabilizing transformation of the data was used if necessary.

[e] If the incidence is not significant, but a significant lack of fit occurs, then Fisher's Exact test with a Bonferroni-Holm correction was used.

C. METHODS

1. Observations

Clinical observations were recorded daily except on the days of neurobehavioral evaluations on test Days 1, 8, and 15.

2. Body weights

Animals were weighed prior to dosing on test Day 1 and on test Days 2, 8, and 15. Body weight gain was calculated for test days 1-2, 2-8, 8-15, and 1-15.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal on test Days 1, 2, 8, and 15. Food efficiency was calculated from food consumption and body weight data.

4. Functional observational battery (FOB) and motor activity (MA)

FOB and MA assessments were conducted on all rats prior to exposure (baseline), on test Day 1 approximately 2 hours post-dosing, and on test Days 8 and 15. The experimenter conducting the FOB was unaware of the treatment group of each animal. The parameters evaluated in the FOB assessment are presented below.

Inside the home cage:
- Posture
- Palpebral closure
- Gait/coordination abnormalities

Tremors
Convulsions

During removal from the home cage and handling :
Ease of removal Palpebral closure
Ease of handling Exophthalmus
Vocalizations Lacrimation
Muscle tone Salivation
Piloerection Dehydration
Fur/skin appearance Emaciation
Mucous membranes

In the open field arena:
Posture Arousal
Gait/Coordination Vocalizations
Tremors Diarrhea
Convulsions Polyuria
Muscle spasms/fasciculation Righting Reflex
Respiration Ease Palpebral Closure
Respiration Rate
The number of rearing movements

While in the standard arena:
Approach/touch
Auditory stimulus
Tail pinch

The remainder of FOB testing involved standardized or calibrated devices:
Fore- and hindlimb grip strength
Hindlimb splay
Rectal body temperature
Pupillary constriction with a beam of light.
Presence of diarrhea and polyuria on the cageboards

Motor Activity (MA):
Motor activity sessions were conducted on the same animals, the same day as FOB assessments, following the FOB assessments. Rats were individually tested in one of 30 nominally identical, automated activity monitors (Coulbourn®). Duration of movement and number of movements were evaluated in 6 consecutive blocks of 10 minutes each, as well as for the total 60-minute session.

5. Sacrifice and neuropathology

At termination on test day 17, six rats/group were anesthetized and underwent whole body *in situ* perfusion fixation. Gross examinations were performed on all test animals. The following tissue samples taken from the organs and tissues were saved from all test

groups:

Brain
- Forebrain
- Cerebrum
- Midbrain
- Cerebellum
- Pons
- Medulla

Spinal cord (cervical and lumbar regions)
- Cervical and lumbar dorsal root ganglia
- Dorsal and ventral root fibers

Sciatic nerve, tibial nerve, sural nerve,
Eye (with optic nerve)
Gastrocnemius muscle

Tissues samples from rats in the control and high-dose groups were processed and evaluated microscopically.

6. Positive control data

The study report stated that procedures and data describing the effects of trimethyltin, amphetamine, carbaryl, and acrylamide in this laboratory have been previously documented in 5 separate reports (References: 1,2,3,4, &5).

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred.

B. CLINICAL OBSERVATIONS

No adverse, test substance-related clinical signs of toxicity were observed in any test groups.

C. BODY WEIGHT

Treatment-related effects on body weights were not observed.

D FOOD CONSUMPTION

Cyantraniliprole did not affect food consumption in treated males or females.

E FUNCTIONAL OBSERVATIONAL BATTERY (FOB) AND MOTOR ACTIVITY

Cyantraniliprole did not produce treatment-related effects on any of the parameters of FOB in male or female test rats.

For motor activity, there was a sporadic decrease in the mean duration of movements during

second of six successive 10-minute intervals on test day 1 for high dose males (2000 mg/kg bw/day) (Table 3). The decrease (152±81 sec) was found to be statistically difference from the controls (209±55 sec). Since this decrease was transient and there were no effects on the number of movements of any other 10-minute interval or the total number of movements in treated males and females, this difference was not considered toxicologically significant. Therefore, no compound-related effect on motor activity was observed.

Table 3.
Motor Activity Assessment: Mean Duration of Movement (Sec) for Male Rats

GROUP	DOSAGE (mg/kg)	SUCCESSIVE 10-MINUTE INTERVALS						
		1	2	3	4	5	6	TOTAL
BASELINE								
I	0	330 (55)	205 (68)	109 (126)	28 (53)	12 (22)	12 (11)	696 (267)
III	250	340 (51)	222 (63)	130 (93)	46 (72)	23 (51)	13 (26)	774 (236)
V	1000	333 (44)	201 (79)	83 (81)	42 (73)	35 (58)	23 (36)	716 (235)
VII	2000	317 (61)	213 (78)	135 (66)	53 (75)	33 (65)	23 (33)	772 (194)
DAY 1								
I	0	349 (60)	209 (55)	60 (70)	15 (17)	13 (12)	6 (11)	651 (146)
III	250	358 (60)	212 (85)	55 (49)	18 (33)	21 (32)	13 (23)	676 (117)
V	1000	342 (48)	162 (68)	47 (69)	14 (30)	32 (33)	36 (72)	632 (150)
VII	2000	317 (67)	152 (81)#	56 (56)	27 (50)	7 (7)	9 (14)	568 (176)
DAY 8								
I	0	352 (54)	287 (83)	201 (107)	130 (128)	48 (74)	31 (58)	1048 (360)
III	250	361 (50)	264 (81)	148 (94)	58 (75)	52 (77)	43 (74)	926 (267)
V	1000	354 (50)	266 (68)	163 (79)	70 (87)	54 (80)	66 (104)	973 (305)
VII	2000	342 (67)	222 (82)	155 (76)	100 (84)	43 (62)	21 (47)	882 (227)
DAY 15								
I	0	354 (63)	228 (80)	151 (80)	76 (95)	45 (78)	6 (6)	859 (273)
III	250	354 (68)	223 (94)	141 (92)	125 (123)	90 (109)	46 (84)	979 (479)
V	1000	367 (47)	247 (55)	172 (93)	94 (96)	60 (76)	56 (105)	995 (331)
VII	2000	344 (73)	238 (92)	141 (119)	104 (120)	61 (54)	49 (97)	937 (364)

Data arranged as: Mean (Standard Deviation).
Number in Group (N) = 12.

Statistically significant trend compared to the control at $p < 0.05$ by Jonckheere-Terpstra trend test.

Table excerpted from page 57 of the report.

F. GROSS PATHOLOGY AND NEUROPATHOLOGY

No test substance-related gross pathology effects were observed. Neuropathological evaluation of brain, spinal cord, skeletal muscles, and peripheral nerves did not reveal any test treatment-related changes. However, minimal axonal degeneration was observed in one control male, one 2000 mg/kg bw male, and one 2000 mg/kg bw female. In all cases, only one or two nerve fibers were involved and were not considered compound related.

III. CONCLUSIONS

When cyantraniliprole was administered to test rats in a single gavage dose of 0, 250, 1000, and 2000 mg/kg bw, no treatment-related effects were seen on the following evaluations: survival,

body weight, food consumption, clinical signs, function observation battery, motor activity, gross pathology, and neuropathology. The NOAEL for acute neurotoxicity was 2000 mg/kg bw (highest dose tested) for male and female rats. No LOAEL was established based on the absence of toxicity at any dose level.

This study is fully reliable (acceptable/guideline) and satisfies the data requirements for an acute neurotoxicity study (OPPTS 870.6200).

REFERENCES

1. DuPont Haskell Laboratory (1995). Neurotoxicity Evaluation of Trimethyltin in Rats (Positive Control Study). Unpublished data, HLR 266-95.

2. DuPont Haskell Laboratory (1997). Neurotoxicity Evaluation of Amphetamine in Rats(Positive Control Study). Unpublished data, HL-1997-00686.

3. DuPont Haskell Laboratory (1997). Neurotoxicity Evaluation of Carbaryl in Rats (Positive Control Study). Unpublished data, HL-1997-00361.

4. DuPont Haskell Laboratory (1996). Neurotoxicity Evaluation of Acrylamide in Rats(Positive Control Study). Unpublished data, HLR 293-95.

5. DuPont Haskell Laboratory (2000). Neurotoxicity Evaluation of Carbaryl in Rats (Positive Control Study). Unpublished data, DuPont-3468.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.1.3 Metabolism study – Repeated dose, oral route in rats

IIA 5.1.3/01

Report: Gannon, S.A. (2010); ^{14}C-DPX-HGW86: Disposition in male and female rats during and after multiple dose administration. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.. DuPont-17399, Revision No. 1. Original report completion: July 16, 2009. Report Revision 1 completed: Dec. 02, 2010. MRID 48119951. Unpublished.

Guidelines: U.S. EPA OPPTS 870.7485 (1998)
OECD Section 4 (Part 417) (1984)
JMAFF 59 Nousan Number 4200 (1985)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a repeated dosing metabolism study (MRID 48119951), a mixture of [CN-^{14}C]-cyantraniliprole and [PC-^{14}C]-cyantraniliprole at a 1:1 mg ratio was administered to groups of 3 Sprague-Dawley rats/sex/dose in multiple doses by oral gavage at a dose level of 10 mg/kg bw/day. There were two objectives for this study. The first was to examine the kinetics of ^{14}C-cyantraniliprole during dose administration and the rates of elimination (half-lives) after dose administration. The following tissues were examined: whole blood, plasma, red blood cells, fat, kidneys, liver, and muscle. The second objective was to establish the profile of metabolites in urine and faeces collected for 24 hours after the first, seventh, and last day of dosing. The average daily dose rate was 10.5 mg/kg/day, corresponding to a radioactive dose rate of 3.0 μCi/rat/day.

Concentration (μg equiv/g), tissue:plasma ratio, and percent of dose were calculated for tissues collected from male and female rats at various times during and after exposure. The data on the tissue concentrations and the tissue percent recovery showed that tissue concentration fell rapidly following the end of dosing. The tissue concentration half-lives ranged from 2.6 days in fat to approximately 6 days in whole blood. The tissue:plasma ratio were all less than 1 following the end of the dosing period. The short tissue half-lives and the tissue:plasma ratio indicated that there was very little tissue accumulation.

The cumulative excretion of total radioactivity in urine and faeces was evaluated from Day 1 through Day 20 as both the percent of accumulating dose and the percent of total dose. The accumulating dose in urine, which represents the amount excreted in the urine in a 24 hr period, ranged from 24 to 29% in males and 13 to 20% in females. The total percent of dose eliminated in the urine was 29% in male rats and 20% in female rats. The accumulating dose in feces ranged from 43 to 61% in males and 42 to 62% in females. The total percent of dose eliminated in the faeces was 61% in males and 62% in females.

Metabolites in urine and faeces during and following multiple dosing were the same as those observed in the single oral dose study (MRID 48119949). In most cases there was very little difference observed between metabolite distribution on Day 1, 7, or 14 in urine metabolites. In urine from male rats IN-N7B69 was present at approximately 5% of the dose on Day 1 and 7 but was not detected on Day 14. It was not detected on any day in female rat urine. IN-MYX98 was present only at 0.5% of the administered dose in male urine on Day 1, but was present at significantly higher levels by Day 7 (5% of dose) and Day 14 (3% of dose). In contrast, this same metabolite was present at 7% on Day 1, 11% on Day 7, but only 1% on Day 14. Cyantraniliprole was approximately 5% of the dose in female rat urine on Days 1 and 7, but not detected on Day 14. IN-MLA84 in female urine was less than 1% of the dose for Days 1 and 7, but was 14% of the dose on Day 14. In feces, the increases in IN-MLA84 and in IN-MYX98 occurred over time (IN-MLA84: Day 1, 1%; Day 15, 5% of the dose. IN-MYX: Day 1, 10%; Day 14, 16%). Parent cyantraniliprole was present in male and female feces range from 7% to 19% of the administered dose.

Cyantraniliprole is readily hydroxylated to form IN-N7B69 and IN-MYX98. IN-N7B69 is further metabolized to a glucuronide. Cyantraniliprole undergoes ring closure to generate IN-J9Z38, which is then in turn hydroxylated to form IN-NBC94, its carboxylic acid, and its glucuronide conjugate. IN-MYX98 is also metabolized to the closed-ring metabolite IN-MLA84, which like IN-NBC94, is further oxidized to a hydroxylated metabolite, a carboxylic acid, and the glucuronide of the hydroxyl metabolite. Further, the hydroxylated metabolite IN-MYX98 can be N-dealkylated to form IN-HGW87 as well as being hydroxylated a second time to form bis-hydroxy-cyantraniliprole. Cyantraniliprole can also be hydroxylated on the pyridine ring, followed by a ring closure analogous to the conversion of cyantraniliprole to IN-J9Z38. Cyantraniliprole can also be N-dealkylated and cleaved at the carbonyl bridge to form IN-DBC80.

This study is reliable (acceptable/non-guideline), and it is considered part (repeated dosing) of the metabolism study.

I. MATERIALS AND METHODS

A. MATERIALS

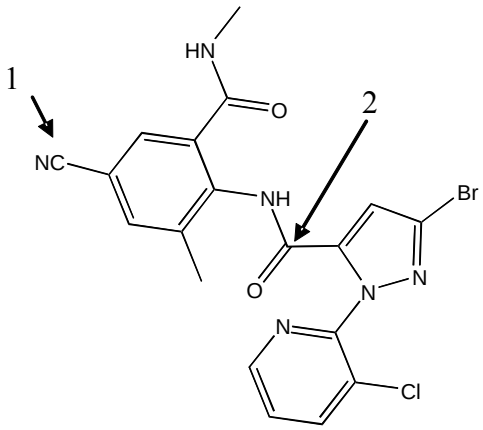
1. Test material:
   Lot/Batch #:
   Purity:
   Description:
   CAS #:
   Stability of test compound:

   Cyantraniliprole technical
   HGW86-141
   93.4%
   Solid, powder
   736994-63-1
   The test substance and major metabolites were found to be stable in urine and faeces extracts over a time interval of one month.

2. Radiolabeled test material:
   Lot/Batch #:

   ^{14}C-cyantraniliprole technical
   [Cyano-^{14}C]-cyantraniliprole, abbreviated as [CN-^{14}C]-cyantraniliprole
   Lot #: 3503-242
   [Pyrazol-Carbonyl-^{14}C]-cyantraniliprole abbreviated as [PC-^{14}C]cyantraniliprole
   Lot #: 3562-048

   Chemical structure /positions of radiolabels:
   1. [CN-^{14}C]-cyantraniliprole
   2. [PC-^{14}C]-cyantraniliprole



   Radio-chemical purity:
   Specific activity:

   [CN-^{14}C]cyantraniliprole: 99%
   [PC-^{14}C]cyantraniliprole: 98.1%
   [CN-^{14}C]cyantraniliprole: 16.95 μCi/mg
   [PC-^{14}C]cyantraniliprole: 17.18 μCi/mg

   Description:
   Stability of test compound:

   Not specified
   The positions of the radiolabels are considered to be stable.

3. Vehicle and/or positive control:

   PEG 400

4. Test animals
   Species:
   Strain:
   Age at dosing:
   Weight at dosing:

   Rat
   Crl:CD®(SD)
   At least 8 weeks
   230.7–247.9 g (males); 157.1–194.3 g (females)

Source:	Charles River Laboratories, Raleigh, NC
Acclimation period:	Minimum of 6 days quarantine
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Rats were housed separately in stainless steel, wire-mesh cages suspended above cage boards.

5. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

February 14, 2007 to July 31, 2007

2. Dosing solution preparation

The 14C-cyantraniliprole was diluted with unlabeled cyantraniliprole to the appropriate specific activity. The dose vehicle (PEG 400) was added and mixed to a clear solution or homogeneous suspension. The dose level (10 mg/kg bw/day) used in this study was the same as the low dose level used in the single dose metabolism study (MRID 49119949; DuPont 16995). All rats were administered a mixture of [cyano-14C]- and [pyrazole carbonyl-14C]- cyantraniliprole at a 1:1 mg ratio. The dosing solution was prepared prior to the first day of use and frozen when not in use. The dose solution was prepared as follows:

Dose Level (mg/kg bw/day)[a]	Dose Volume (mL/kg bw)	Radiochemical Dose (µCi/kg bw)[b]	Specific Activity in Dose (µCi/mg)	Chemical Concentration (mg/mL)	Radiochemical Concentration (µCi/mL)
10	2	20	4	5	10

a 0.25 kg body weight/rat
b 5 µCi/rat/day

Table excerpted from page 19 of the study report

3. Study design (Tables 1 and 2)

The design of the in-life phase of this study was based on the principle of superposition. The concentration time courses of ^{14}C residues in male and female rat plasma following

single oral gavage administration were modelled by adding the 0 to 120 hour data to itself at 24 hour intervals. The predicted superposition indicates that the kinetics in plasma should be saturated by 5 days of dose administration in males and by 10 days in males. The single dose study showed that female rats have higher plasma and tissue ^{14}C residue concentrations than male rats. For this reason, the disposition of cyantraniliprole during and after multiple dose administration was studied in female rats as shown in Table 1. Male rats were included for comparison with female rats for tissue distribution (Groups 4 and 5) and material balance and tissue distribution (Groups 7 and 8). All test rats received the test material by gavage.

Table 1. Study design

Number of rats and sex [a]									
Day of experiment	**Group 1 3F**	**Group 2 3F**	**Group 3 3F**	**Group 4 3M**	**Group 5 3F**	**Group 6 3F**	**Group 7 3M**	**Group 8 3F**	**Group 9 3F**
1	D	D	D	D	D	D	D	D	D
2	D	D	D	D	D	D	Um,Fm, D	Um,Fm, D	D
3	D	D	D	D	D	D	U,F,D	U,F,D	D
4	D	D	D	D	D	D	U,F,D	U,F,D	D
5	S	D	D	D	D	D	U,F,D	U,F,D	D
6		D	D	D	D	D	U,F,D	U,F,D	D
7		D	D	D	D	D	U,F,D	U,F,D	D
8		D	D	D	D	D	Um,Fm, D	Um,Fm, D	D
9		S	D	D	D	D	U,F,D	U,F.D	D
10			D	D	D	D	U,F,D	U,F.D	D
11			D	D	D	D	U,F,D	U,F.D	D
12			S	D	D	D	U,F,D	U,F.D	D
13				D	D	D	U,F,D	U,F.D	D
14				D	D	D	U,F,D	U,F.D	D
15				Sa	Sa		Um,Fm, D	Um,Fm, D	
16							U,F	U,F	
17						S	U,F	U,F	
18							U,F	U,F	
19							U,F	U,F	
20							U,F	U,F	
21							U,F,Sa, CW,FR	U,F,Sa, DW,FR	
26									S

[a] bw = Body weight

CW = Cage wash (collected throughout study from excreta separator and from entire unit at the end of the study as composite sample)

D = Dose administration 10 mg/kg bw/day, approximately 5 μCi/rat/day

F = Faeces, Fm – faeces for metabolic profiling

FR = Feed residue (collected throughout study as composite sample)

S = Sacrifices with collection of selected tissues (whole blood, plasma, red blood cells, liver, kidney, fat, and muscle)

Sa = Sacrifices with full tissue (describe below) and carcass collection

U = Urine; Um = Urine for metabolite profiling

Rats in Groups 7 and 8 were placed in glass or plastic metabolism units starting the evening before dose administration for collection of urine and feces at 24 hour intervals and until 7 days after the last daily dose administration (experiment day 21).

4. Tissue sample

The tissues shown in Table 2 were collected from male and female rats at various times during and after exposure, and residue concentrations were measured. More collections were done in female rats because female rats exhibited a higher systemic availability following a single oral gavage in a previous study (MRID 49119949). In male rats, tissues were collected on Day 15 and Day 21 (one day and 7 days after last dose). Tissues were collected from female rats on Days 5, 9, 12, 15, 17, 21, and 26. All tissues were collected from male and female animals of Day 15 and Day 21, but a more limited set of tissues was collected from female rats on the other collection days.

Table 2. Tissue samples collected

blood (plasma & RBC)a	Fat	Liver
Kidney	Muscle	Heart
Lung	Testes	Ovaries
Uterus	Bone and bone marrow[a]	Brain
Spleen	Adrenal	Pituitary
G.I. tract and content[a]	Pancreas	Skin sample
Thyroid	Thymus	Bladder[b]

[a] Analyzed separately for 14C content
[b] Urine in the bladder at the time of sacrifice was aspirated and placed in the vial used for the last 24 hour collection interval
Information excerpted from page 21 of the study report.

After collection, tissue samples were stored at approximately $\leq 10\ ^0C$ until processing and analysis. The remaining carcass for rats in Groups 4, 5, 7, and 8 were homogenized and assayed for the determination of material balance.

4. **Clinical Observations and Mortality**

Cage-site examinations to detect moribund or dead rats and abnormal behavior and/or appearance were conducted at least once daily throughout the study.

5. Identification and quantitation of metabolites in various tissues

Metabolites were confirmed by comparison of LC/MS full scan and daughter ion scans of each component with scans for the reference standards. The mass spectral analysis in urine or faeces was facilitated by the presence of a characteristic ion cluster containing one bromine and one chlorine. The molecular ion cluster had relative intensities of (0.77):(1.0):(0.24) respectively for (MH^+):$(M+2)H^+$:$(M+4)H^+$ when the 2 halogens were

present in the metabolites. Partial structural information was provided for the unidentified components in urine and faeces when possible.

Urine
The urine samples were pooled across the individual animals for a given collection interval to obtain a kinetic representation at Day 1, Day 7, and Day 14. Individual components greater than 0.5% of the administered dose are labeled on the radio-chromatograms. Cyantraniliprole and its metabolites were identified by comparing LC/MS full scan spectra, daughter ion spectra, and retention times for the corresponding reference standards. Where reference standards were not available, tentative structures were assigned based on mass spectral data.

Feces
The faeces samples were pooled across the individual animals for a given collection interval to obtain a kinetic representation at Day 1, Day 7, and Day 14. Cyantraniliprole and its metabolites were identified by comparing LC/MS full scan spectra, daughter ion spectra, and retention times for the corresponding reference standards. Where reference standards were not available, tentative structures were assigned based on mass spectral data.

Plasma
Plasma samples were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Selected plasma samples were analyzed for parent compound and metabolites by LC-MS.

Tissues and Red Blood Cells
Tissues and red blood cells were homogenized and aliquots combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total radioactivity present in the tissue.

Cage Rinses
Aliquots of cage rinses were analyzed in triplicate for ^{14}C by LSC.

II. RESULTS AND DISCUSSION

A. PILOT STUDY

The percent recovery and tissue concentration data showed that female rats retained a greater proportion of ^{14}C residues than male rats. The declines of the plasma and tissue ^{14}C residue concentrations at high and low doses were generally similar. These observations were consistent with the shorter elimination half-life in male rats compared with female rats.

B. STORAGE STABILITY

The test substance and major metabolites (*i.e.*, those found to be greater than 5% of the dose) were found to be stable in urine and faeces extracts over a time interval of one month.

C. MAIN STUDY

Distribution

The tissue concentration data indicated that the highest concentration was found on day 15 (the day after exposure termination) and in whole blood, liver, fat, and plasma (Tables 3 & 4). The tissue concentrations and the tissue percent recovery demonstrated that distributed cyantraniliprole in various tissue fell rapidly the day after dosing (Day 15). The percent recovery data showed that there was minimal amount of the administered dose remaining at or after day 21, and there was negligible tissue accumulation in the test rats (Table 5). Tissue which contained highest concentrations was plasma followed by whole blood, fat, liver, pituitary, adrenal, and gastrointestinal content (Tables 5a and 5b). The study report also presented the data on tissue: plasma ratios for various tissue, and the ratios were less than 1.0 (Table 6). This also supported the observation that there was very little tissue accumulation. Since tissues were collected at many time points in female rats, it was possible to calculate tissue elimination half-lives; these half-lives range from 2.6 days in fat to approximately 6 days in whole blood (Table 7).

Table 3. Time course of ^{14}C-residue concentration (µg equiv/g) in male rats after 14-day oral gavage dosing.

		Concentration		Tissue:Plasma Ratio		Percent of Dose	
		Mean	SD	Mean	SD	Mean	SD
whole blood	Day 15	7.74	1.52	0.52	0.02	0.421	0.076
whole blood	Day 21	3.41	2.19	0.55	0.03	0.188	0.125
fat	Day 15	2.35	0.53	0.16	0.05	0.158	0.037
fat	Day 21	0.38	0.23	0.06	0.01	0.026	0.016
kidney	Day 15	4.30	1.23	0.29	0.04	0.029	0.008
kidney	Day 21	1.36	0.69	0.24	0.07	0.009	0.004
liver	Day 15	6.35	1.69	0.43	0.05	0.232	0.055
liver	Day 21	1.83	0.75	0.33	0.07	0.068	0.029
muscle	Day 15	0.95	0.44	0.06	0.02	0.295	0.133
muscle	Day 21	0.36	0.22	0.06	0.01	0.114	0.072
plasma (terminal)	Day 15	14.74	2.51	1.00	NA	0.029	0.008
plasma (terminal)	Day 21	6.12	3.86	1.00	NA	0.010	0.007
rbc (terminal)	Day 15	2.97	0.32	0.20	0.02	0.008	0.001
rbc (terminal)	Day 21	1.52	0.77	0.26	0.06	0.004	0.002

Table excerpted from page 35 of the study report.

Table 4. Time course of ^{14}C-residue concentration (µg equiv/g) in female rats after 14-day oral gavage dosing.

		Concentration		Tissue:Plasma Ratio		Percent of Dose	
		Mean	SD	Mean	SD	Mean	SD
whole blood	Day 5	7.74	0.61	0.62	0.004	1.268	0.074
whole blood	Day 9	17.10	2.80	0.56	0.02	1.417	0.248
whole blood	Day 12	24.46	2.36	0.57	0.02	1.487	0.159
whole blood	Day 15	30.86	4.04	0.53	0.17	1.517	0.127
whole blood	Day 17	24.93	6.93	0.58	0.04	1.264	0.338
whole blood	Day 21	10.96	5.65	0.57	0.02	0.54	0.282
whole blood	Day 26	8.71	7.34	0.55	0.02	0.441	0.367
fat	Day 5	31.50	2.21	2.52	0.21	4.503	0.34
fat	Day 9	35.44	3.91	1.17	0.06	2.559	0.328
fat	Day 12	34.59	7.01	0.81	0.20	1.826	0.33
fat	Day 15	44.99	8.46	0.78	0.29	1.925	0.281
fat	Day 17	19.24	4.38	0.48	0.21	0.853	0.204
fat	Day 21	5.11	1.80	0.32	0.17	0.219	0.078
fat	Day 26	2.41	0.77	0.25	0.18	0.107	0.037
kidney	Day 5	4.51	0.52	0.36	0.02	0.090	0.005
kidney	Day 9	6.95	0.58	0.23	0.02	0.068	0.003
kidney	Day 12	9.39	0.92	0.22	0.01	0.068	0.010
kidney	Day 15	10.36	1.48	0.18	0.06	0.067	0.013
kidney	Day 17	7.79	2.13	0.18	0.02	0.041	0.008
kidney	Day 21	4.08	2.30	0.21	0.04	0.027	0.016
kidney	Day 26	2.39	1.73	0.18	0.07	0.015	0.011
liver	Day 5	15.30	1.67	1.22	0.08	1.680	0.287
liver	Day 9	19.92	1.62	0.66	0.05	1.047	0.17
liver	Day 12	25.40	1.41	0.60	0.07	0.883	0.272
liver	Day 15	30.72	5.47	0.53	0.19	1.012	0.157
liver	Day 17	15.07	1.33	0.36	0.05	0.490	0.014
liver	Day 21	6.50	2.51	0.37	0.13	0.235	0.107
liver	Day 26	4.31	2.57	0.36	0.19	0.133	0.074
muscle	Day 5	2.18	0.59	0.17	0.03	2.040	0.507
muscle	Day 9	2.68	0.62	0.09	0.01	1.269	0.317
muscle	Day 12	3.15	0.36	0.07	0.01	1.093	0.104
muscle	Day 15	4.65	1.62	0.08	0.04	1.297	0.389
muscle	Day 17	2.73	0.58	0.06	0.002	0.791	0.158
muscle	Day 21	1.29	0.74	0.07	0.01	0.364	0.211
muscle	Day 26	0.88	0.71	0.06	0.01	0.254	0.204

Table 4 (Continued). Time course for 14C-residue concentration (µg equiv/g) in female rats after 14-day oral gavage dosing.

		Concentration		Tissue:Plasma Ratio		Percent of Dose	
		Mean	SD	Mean	SD	Mean	SD
plasma (terminal)	Day 5	12.53	1.05	1.00	NA	0.145	0.009
plasma (terminal)	Day 9	30.57	4.83	1.00	NA	0.151	0.023
plasma (terminal)	Day 12	42.80	2.94	1.00	NA	0.169	0.031
plasma (terminal)	Day 15	60.11	9.81	1.00	NA	0.161	0.059
plasma (terminal)	Day 17	42.68	9.47	1.00	NA	0.139	0.067
plasma (terminal)	Day 21	19.42	10.22	1.00	NA	0.046	0.031
plasma (terminal)	Day 26	16.05	13.78	1.00	NA	0.046	0.04
rbc (terminal)	Day 5	2.91	0.52	0.23	0.03	0.034	0.006
rbc (terminal)	Day 9	6.86	1.78	0.22	0.03	0.046	0.013
rbc (terminal)	Day 12	7.46	0.95	0.18	0.03	0.035	0.005
rbc (terminal)	Day 15	10.43	0.54	0.18	0.02	0.035	0.003
rbc (terminal)	Day 17	8.49	1.99	0.20	0.03	0.031	0.009
rbc (terminal)	Day 21	5.11	2.12	0.28	0.05	0.017	0.006
rbc (terminal)	Day 26	2.57	1.64	0.20	0.08	0.008	0.006

Data excerpted from pages 36 and 37of the study report

Table 5. Percent recovery in male and female rats – days 15 and 21 after 14-day multiple oral dosing 10 mg/kg bw/day radiolabeled cyantraniliprole

	Male		Female	
Tissue	**Day 15**	**Day 21**	**Day 15**	**Day 21**
Skin	0.65	0.28	2.35	0.64
Whole blood	0.42	0.19	1.52	0.54
Bone marrow	0.00	0.001[b]	0.00	<0.001[b]
Brain	0.00	0.001[a]	0.01	0.002
Fat	0.16	0.03	1.93	0.22
RBC (terminal)	0.01	0.00	0.04	0.02
Heart	0.01	0.00	0.04	0.01
Lungs	0.02	0.01	9,08	0.03
Spleen	0.00	0.001[b]	0.01	0.00
Liver	0.23	0.07	1.01	0.24
Kidney	0.03	0.01	0.07	0.03
G.I. tract	0.06	0.01	0.30	0.07
G.I. contents	0.78	0.06	1.48	0.19
Pituitary	<0.001 [a]	<LOQ[c]	0.00	<0.001[a]
Thyroid	0.00 [a]	<0.001[a]	0.00	0.001[b]
Thymus	0.00	0.00	0.01	0.01
Testes	0.01	0.01	--	—
Ovaries	--	—	0.01	0.002
Pancreas	0.01	0.00	0.03	0.01
Adrenals	0.00	<0.001[a]	0.01	0.00
Plasma (terminal)	0.03	0.01	0.16	0.05
Uterus	--	—	0.02	0.01
Muscle	0.30	0.11	1.30	0.36
Bladder	0.00	<0.001[b]	0.01	0.00
Bone	0.06	0.03	0.17	0.05

[a] n = 1
[b] n = 2
[c] All samples below LOD/Q

Note: Some values are greater that the LOQ but less than <0.001%. The values are reported as <0.001 for consistency with significant figures used in the table.

Data excerpted from pages 43 and 44 of the study report.

Table 5a

Tissue concentration in male rats - Day 15 and Day 21 - 1 and 7 days after 14-day multiple oral dosing 10 mg/kg bw/day

	Concentration (ug equiv/g tissue)			
	Day 15		Day 21	
Sample	Mean	SD	Mean	SD
skin	4.36	0.478	1.895	0.926
whole blood	7.736	1.518	3.414	2.193
bone marrow	1.855	0.179	1.013[b]	NA
brain	0.26	0.059	0.139[a]	NA
fat	2.354	0.533	0.383	0.225
rbc (terminal)	2.971	0.318	1.517	0.77
heart	3.126	0.707	1.218	0.689
lungs	4.437	0.599	2.313	1.709
spleen	1.435	0.327	0.543	0.337
liver	6.348	1.69	1.827	0.754
kidney	4.304	1.234	1.362	0.692
G.I. tract	3.263	1.188	0.775	0.406
G.I. contents	13.514	3.997	1.167	0.334
pituitary	9.34[a]	NA	NA[c]	NA
thyroid	12.457	8.548	2.705[a]	NA
thymus	2.152	0.942	0.699	0.296
testes	1.888	0.363	0.833	0.52
pancreas	1.832	0.225	0.72	0.494
adrenals	7.923	1.442	3.43	1.528
plasma (terminal)	14.744	2.514	6.116	3.862
muscle	0.949	0.44	0.363	0.219
bladder	5.203	2.495	1.838[b]	NA
bone	1.104	0.162	0.446	0.262

a n = 1
b n = 2
c all samples below LOD/Q

Table excerpted from page 39 of the study report.

Table 5b
Tissue concentration in female rats - Day 15 and Day 21 - 1 and 7 days after 14-day multiple oral dosing 10 mg/kg bw/day

	Concentration (ug equiv/g tissue)			
	Day 15		Day 21	
Sample	Mean	SD	Mean	SD
skin	17.628	1.84	4.82	1.955
whole blood	30.862	4.035	10.961	5.648
bone marrow	7.946	1.438	3.361[b]	NA
brain	1.674	0.179	0.347	0.161
fat	44.989	8.455	5.109	1.804
rbc (terminal)	10.429	0.54	5.111	2.115
heart	12.805	1.59	4.204	2.085
lungs	19.12	1.505	6.29	3.386
spleen	5.843	0.854	1.851	0.971
liver	30.724	5.468	6.497	2.51
kidney	10.364	1.479	4.082	2.296
G.I. tract	15.005	3.045	2.9	1.23
G.I. contents	31.885	3.563	2.993	0.759
pituitary	29.143	6.983	12.313[a]	NA
thyroid	21.224	8.456	10.396	9.436
thymus	9.022	4.82	2.984	2.003
ovaries	19.864	2.862	5.26	2.147
pancreas	11.577	2.072	2.65	1.066
adrenals	28.815	3.23	9.095	3.017
plasma (terminal)	60.107	9.812	19.418	10.224
uterus	12.565	1.613	5.157	3.439
muscle	4.646	1.621	1.293	0.743
bladder	21.375	7.017	5.145	2.53
bone	3.243	1.195	1.06	0.39

a n = 1
b n = 2
Table excerpted from page 40 of the study report

Table 6.
Tissue:plasma ratio in male rats - Day 15 and Day 21 after 14-day multiple dosing 10 mg/kg bw/day

	Tissue:Plasma Ratio			
	Day 15		Day 21	
Sample	Mean	SD	Mean	SD
skin	0.300	0.050	0.351	0.174
whole blood	0.523	0.018	0.553	0.034
bone marrow	0.127	0.012	0.138[b]	NA
brain	0.018	0.002	0.013[a]	NA
fat	0.164	0.052	0.063	0.009
rbc (terminal)	0.203	0.017	0.265	0.055
heart	0.211	0.019	0.206	0.020
lungs	0.304	0.039	0.363	0.039
spleen	0.097	0.009	0.089	0.002
liver	0.427	0.047	0.327	0.069
kidney	0.289	0.039	0.240	0.067
G.I. tract	0.217	0.044	0.132	0.013
G.I. contents	0.905	0.117	0.219	0.069
pituitary	0.634[a]	NA	<LOQ[c]	NA
thyroid	0.796	0.500	0.258[a]	NA
thymus	0.142	0.048	0.124	0.024
testes	0.128	0.003	0.136	0.010
pancreas	0.125	0.014	0.115	0.006
adrenals	0.551	0.161	0.616	0.174
plasma (terminal)	1.000	NA	1.000	NA
muscle	0.063	0.020	0.060	0.005
bladder	0.350	0.159	0.2352	NA
bone	0.076	0.015	0.074	0.004

a n = 1
b n = 2
c all samples below LOD/Q

Data excerpted from page 41 of the study report.

Table 6a. Tissue:plasma ratio in female rats - Day 15 and Day 21 - 1 and 7 days after 14-day multiple oral dosing 10 mg/kg bw/day

	Tissue:Plasma Ratio			
	Day 15		Day 21	
Sample	Mean	SD	Mean	SD
skin	0.302	0.085	0.269	0.064
whole blood	0.531	0.165	0.570	0.019
bone marrow	0.136	0.038	0.135[a]	NA
brain	0.029	0.008	0.019	0.003
fat	0.778	0.285	0.316	0.174
rbc (terminal)	0.176	0.021	0.280	0.049
heart	0.220	0.067	0.223	0.025
lungs	0.327	0.079	0.326	0.024
spleen	0.101	0.033	0.097	0.010
liver	0.531	0.191	0.375	0.132
kidney	0.178	0.056	0.214	0.035
G.I. tract	0.260	0.100	0.159	0.029
G.I. contents	0.537	0.079	0.179	0.074
pituitary	0.484	0.080	0.436[b]	NA
thyroid	0.343	0.093	0.462	0.241
thymus	0.162	0.114	0.144	0.029
ovaries	0.342	0.111	0.290	0.057
pancreas	0.200	0.070	0.146	0.028
adrenals	0.493	0.135	0.540	0.226
plasma (terminal)	1.000	NA	1.000	NA
uterus	0.215	0.062	0.253	0.048
muscle	0.082	0.044	0.065	0.005
bladder	0.376	0.191	0.272	0.022
bone	0.057	0.030	0.060	0.014

a n = 1
b n = 2
c all samples below LOD/Q
Data excerpted from page 42 of the study report.

Table 7. Pharmacokinetic analysis of female rat 14C residue concentration time course data from multiple oral gavage study

Tissue	Half-life (days)	Tmax (days)	C_{max} (µg equiv/g)	AUC_{inf} (µg equiv days/g)
Fat	2.6	15	45.0	577.9
Kidney	5.1	15	10.4	164.1
Liver	4.0	15	30.7	401.6
Muscle	4.6	15	4.7	62.3
Plasma	5.6	15	60.1	827.7
Red Blood Cells	5.4	15	10.4	160.5
Whole Blood	5.7	15	30.9	462.7

Data excerpted from page 38 of the study report.

Elimination

The percent of dose eliminated in the feces was clearly more than that eliminated in urines for males and females (Figure1). The accumulating dose in urine, which represents the amount excreted in the urine in a 24 hr period, ranged from 24 to 29% in males and 13 to 20% in females. The total percent of dose eliminated in the urine was 29% in male rats and 20% in female rats (Table 8). The accumulating dose in faeces ranged from 43 to 61% in males and 42 to 62% in females. The total percent of dose eliminated in the faeces was 61% in males and 62% in females.

Figure 1. Elimination of 14C-cyantraniliprole in urine and feces.
(Excerpred from page 59 of the report).

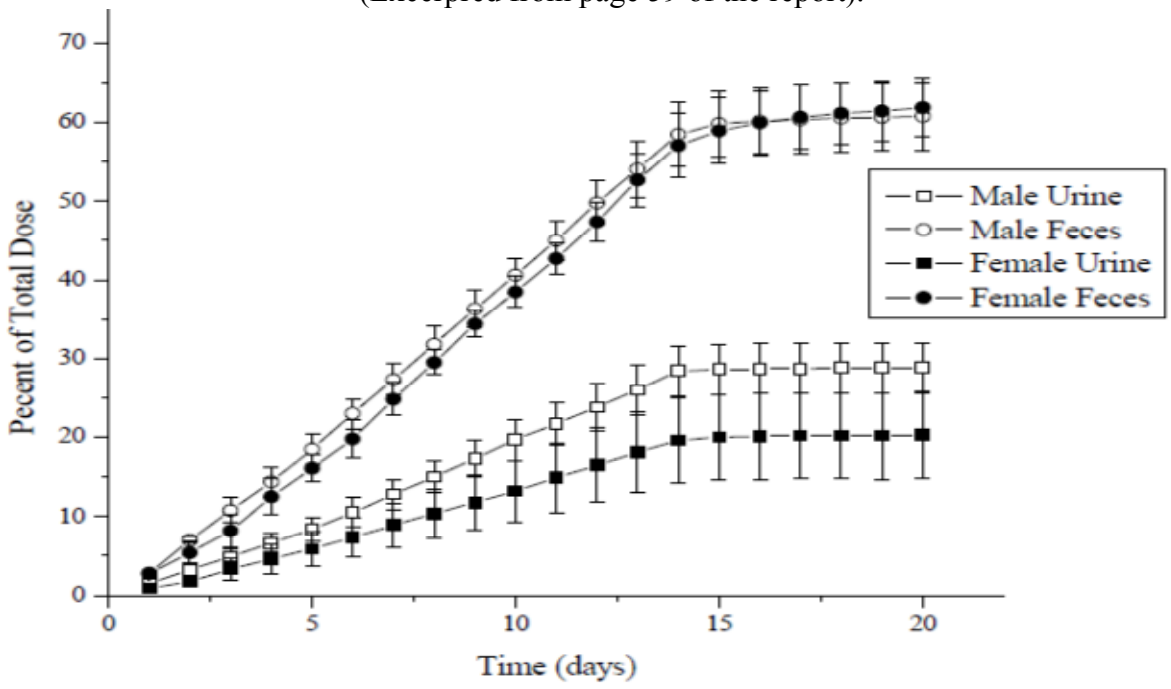


Table 8. Percent of the administered dose eliminated and remained in the tissues.

	Males	Females
Urine	29 ±3	20±6
Feces	61±4	62±4
Tissues[a]	0.8±0.4	3±1
Cage Wash	3±1	5±1
Total	93±9	89±11

[a]: Muscle, bone, and fat scaled the entire animal. Do not include carcass.
Data excerpted from page 47 of the study.

Metabolites

Radio-chromatography and mass spectral analysis suggested the presence of at least 10 metabolites comprising approximately 75% of the administered dose eliminated in urine.
The metabolites identified in urine during multiple dosing were all observed in the single dose ADME study (MRID 48119949) as well. In most cases there was very little difference observed between metabolite distribution on Day 1, 7, and 14 (Table 9). In urine from male rats, IN-N7B69 was present at approximately 5% of the dose on Day 1 and 7 but was not detected on Day 14. It was not detected on any day in female rat urine. IN-MYX98 was present only at 0.5% of the administered dose in male urine on Day 1 but was present at significantly higher levels by Day 7 (5% of dose) and Day 14 (3% of dose). In contrast, in female rats, this same metabolite was present at 7% on Day 1, 11% on Day 7, but only 1% on Day 14. Cyantraniliprole was approximately 5% of the dose in female rat urine on Days 1 and 7, but not detected on Day 14. In female urine, IN-MLA84 was less than 1% of the dose for Days 2 and 7, but was 14% of the dose on Day 14.

Table 9. Summary of ^{14}C-DPX-HGW86 and metabolites as percent of dose in urine during multiple dose administration

	Male			**Female**		
	Day 1	**Day 7**	**Day 14**	**Day 1**	**Day 7**	**Day 14**
IN-DBC80	6.54	4.68	6.36	0.66	1.30	1.52
IN-N7B69	5.40	4.91	ND	ND	ND	ND
IN-NBC94 Glucuronide	ND	ND	1.48	ND	ND	0.54
IN-MYX98	0.49	5.02	3.29	7.37	10.85	1.19
IN-HGW87	ND	ND	ND	0.74	0.71	ND
Cyantraniliprole	1.55	1.34	ND	5.08	4.48	ND
IN-NBC94	1.39	3.12	7.95	ND	ND	1.30
m/z 485	2.37	2.68	ND	ND	ND	ND
IN-MLA84	0.49	ND	1.91	0.66	0.83	14.29
IN-J9Z38	ND	ND	0.74	0.66	0.47	0.76
Unidentified	6.13	5.46	6.68	NA	NA	NA
Total	24.37	27.21	28.41	15.15	18.64	19.59

Data excerpted from page 49 of the study report.

Radio-chromatography and mass spectral analysis suggested the presence of at least 14 metabolites comprising approximately 80% of the administered dose eliminated in faeces. The metabolites identified in faeces during multiple dosing were all observed in the single dose ADME study (1) as well. In most cases there was very little difference observed between metabolite distribution on Day 1, 7, or 14. The most notable differences both occur in the female rat faeces samples. There is an increase in both IN-MLA84 (Day 1, 1%; Day 14, 5% of dose) and in IN-MYX98 (Day 1, 10%; Day 14, 16%) as percent of the dose (Table 10). Parent cyantraniliprole was present in male and female feces range from 7% to 19% of the administered dose.

Table 10. Summary of ^{14}C-DPX-HGW86 and metabolites as percent of dose in faeces during multiple dose administration

	Male			Female		
	Day 1	**Day 7**	**Day 14**	**Day 1**	**Day 7**	**Day 14**
Bis HO-HGW86	2.37	5.87	4.55	ND	ND	ND
IN-DBC80	3.50	3.62	2.27	ND	ND	ND
IN-N7B69	1.92	3.81	4.04	0.55	2.79	3.65
IN-MYX98	**9.07**	**9.88**	**10.66**	**10.10**	**12.96**	**16.37**
IN-HGW87	1.99	0.98	1.10	1.22	2.02	2.14
HO-Pyr-HGW86	ND	ND	ND	2.00	1.71	2.05
Cyantraniliprole	**7.42**	**11.05**	**9.84**	**18.97**	**16.11**	**13.54**
IN-NBC94	2.68	3.32	3.13	0.89	2.88	3.41
m/z 344	0.82	0.68	0.63	0.89	0.58	1.27
Isomer of IN-MYX98	ND	ND	ND	0.55	0.99	0.97
IN-J9Z38 Acid	3.37	7.24	6.90	ND	ND	ND
IN-MLA84	0.69	1.81	1.57	1.11	2.47	5.12
MLA84 Acid	0.41	ND	ND	ND	ND	ND
IN-J9Z38	0.65	0.44	0.39	3.16	1.89	1.80
Unidentified	**8.42**	**9.29**	**13.44**	**5.99**	**7.47**	**6.77**
Total	43.33	57.98	58.50	45.44	51.87	57.10

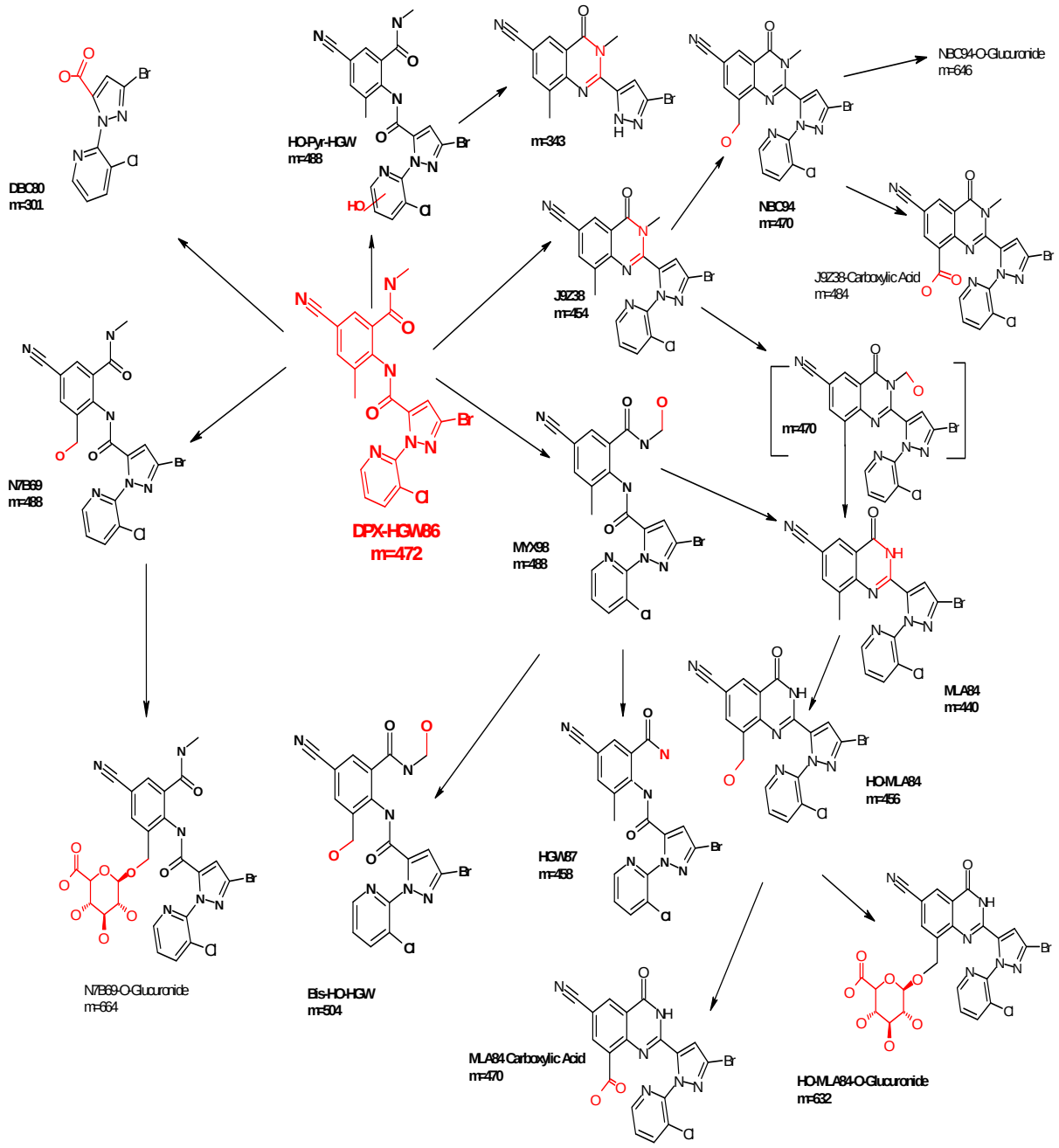
Data excerpted from page 52 of the study report.

No match was found for nine of the reference standards including IN-F6L99, IN-JCZ38, IN-JSE76, IN-K5A77, IN-K5A78, IN-K5A79, IN-K7H19, IN-N5M09, and IN-PLT97.

The proposed metabolic pathway in rats is presented in Figure 2 and can be summarized as follows: Cyantraniliprole is readily hydroxylated to form IN-N7B69 and IN-MYX98. IN-N7B69 is further metabolized to a glucuronide. Cyantraniliprole undergoes ring closure to generate IN-J9Z38, which is then in turn hydroxylated to form IN-NBC94, its carboxylic acid, and its glucuronide conjugate. IN-MYX98 is also metabolized to the closed-ring metabolite IN-MLA84, which like IN-NBC94, is further oxidized to a hydroxyl, a carboxylic acid, and the glucuronide of the hydroxyl metabolite. Further, the hydroxylated metabolite IN-MYX98 can be N-dealkylated to form IN-HGW87 as well as being hydroxylated a second time to form the bis-hydroxy-cyantraniliprole. Cyantraniliprole can also be hydroxylated on the pyridine ring, followed by a ring closure analogous to the conversion of

cyantraniliprole to IN-J9Z38. Cyantraniliprole can also be N-dealkylated at the carbonyl bridge to form IN-DBC80.

Figure 2
Proposed metabolic pathway of cyantraniliprole in the rat: Repeated dose study



III. CONCLUSION

The data show that the tissue concentrations and the tissue percent recovery fall rapidly following the end of dosing and the tissue half-lives range from 2.6 days in fat to approximately 6 days in whole blood. The tissue:plasma ratio were all less than 1 following the end of the dosing period. The short tissue half-lives and the tissue:plasma ratio indicated that there was very little tissue accumulation.

The cumulative excretion of total radioactivity in urine and faeces was evaluated from Day 1 through Day 20 as both the percent of accumulating dose and the percent of total dose. The accumulating dose in urine ranged from 24 to 29% in males and 13 to 20% in females. The total percent of dose eliminated in the urine was 29% in male rats and 20% in female rats. The accumulating dose in faeces ranged from 43 to 61% in males and 42 to 62% in females. The total percent of dose eliminated in the faeces was 61% in males and 62% in females. The overall percent recovery was 93% in males and 89% in females with the majority of the dose eliminated in faeces. The amount remaining in the tissues at sacrifice was very small (0.8% in males and 2.5% in females). Metabolites in urine and faeces during and following multiple dosing were similar as those observed in the single oral dose study (MRID 48119949).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.4 Oral 1-year toxicity (dog)

IIA 5.3.4/01

Report: Luckett, E.M. (2010); DPX-HGW86 technical: Chronic toxicity 1-year feeding study in dogs. MPI Research, Inc., Mattawan, Michigan, USA. Laboratory Report No.: 125-056; DuPont-19180. August 25, 2010. MRID 48119960. Unpublished.

IIA 5.3.4/02

Report: Mawn, M.P. (2010); DPX-HGW86 technical: Chronic toxicity 1-year feeding study in dogs. DuPont Haskell Laboratories; MPI Research, Inc., Newark, Delaware, USA; Mattawan, Michigan, USA. Laboratory Report No.: 125-056. DuPont-19180, Supplement No. 1. September 7, 2010. MRID 48208427. Unpublished.

Guidelines: OPPTS 870.4100
OECD 452,
JMAFF No. 12-Nousan-8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 1-year feeding study (MRID 48119960), cyantraniliprole (94.5%; HGW86-230) was administered in the diet to groups of beagle dogs (4/sex/group) at concentrations of 0, 40, 200, 1000, and 5000 ppm (male: 0, 1, 6, 27, and 144 mg/kg bw/day; females: 0, 1, 6, 27, and 133 mg/kg bw/day) for 364 consecutive days. The 5000 ppm group consisted of 7 dogs/sex, with 3 male and 3 females planned for the recovery study; the recovery animals received 5000 ppm (124 and 135 mg/kg bw/day for male and female, respectively) for 12 weeks and then placed on the control diet for the remainder duration of the study. However, one male of the 5000 ppm group was sacrificed *in extremis* on day 80 reducing the number of dogs in the recovery group to 2 males and 3 females. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, compound consumption, clinical signs, neurobehavioral observations, hematology, coagulation, clinical chemistry, urinalysis, ophthalmology, organ weights, and gross and microscopic pathology. In addition, the blood samples were collected on week 39, analyzed and quantified for the parent compound and metabolites (IN-JCZ38, IN-K7H19, IN-K5A77, IN-JSE76, IN-K5A78, IN-K5A79, IN-PLT97, and IN-NXX70) (MRID 48208427).

One 5000 ppm male was euthanized *in extremis* on Day 80. The need for euthanasia was considered to be due to complications associated with arteritis. This death may be test article related as arteritis was observed in other dogs on this study in the 1000 and 5000 ppm groups (and in one control dog), and at 10000 ppm in a previous 90-day study with this test article (MRID 48119948). A 5000 ppm female was also euthanized on day 176, due to clinical signs attributed to septicaemia from unknown cause, which was not attributed directly to test article exposure. Over the entire dosing period, main study animals at 5000 ppm gained less body

weight than control (35% less in males and 92% less in females).

Treatment-related increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT), and decreases in total protein and albumin were observed in male and female animals at dietary concentrations of 1000 and 5000 ppm. Gamma glutamyltransferase (GGT) was also increased in the 5000 ppm male and female groups. At the 1000 and 5000 ppm concentrations, the clinical chemistry changes were associated with evidence of degenerative and inflammatory changes in the liver and, in a few animals, with minimal cholestasis. Test article-related macroscopic observations were observed in one female each at 1000 ppm and 5000 ppm and consisted of discoloration (tan) and/or irregular surface of the liver. Males and females at 1000 ppm and 5000 ppm had increased group mean liver (with gall bladder) weights (absolute, relative to body weight and to brain weight), that were associated with degenerative and inflammatory microscopic changes in the liver Three males and one female at 5000 ppm had minimal to mild hyperplasia of the mucosa of the gall bladder. These clinical and histopathology changes were considered to be test article related and adverse. Liver weights were also increased in the 40 and 200 ppm males and in 200 ppm females, but the increase in liver weight at 200 ppm were accompanied by statistically significant increase in alkaline phosphatase and decreased albumin level. In the recovery group, these effects were not seen in males or females; however, the finding in the recovery group should not be interpreted as to demonstrate the effects seen in the 1-year treated dogs were reversible because the dogs in recovery group were treated with cyantraniliprole for only 12 weeks.

Test article-related increases in the incidence of arteritis, particularly in coronary arteries, were observed in males at 1000 ppm (3/4) and 5000 ppm (2/4). The increase in the incidence of arteritis was also seen in the 90-day dog study with this test article (MRID 48119948). Increased thyroid/parathyroid weights were observed in 5000 ppm males and were considered possibly test article related, but not adverse, as there were no associated microscopic pathology effects.

The only detectable analyte in plasma was parent cyantraniliprole and there was no apparent sex difference in plasma concentration for any analyte in any treatment groups. At the week 39, the plasma levels of cyantranliprole were 62 µg/mL and 57 µg/mL in the 5000 ppm males and females, respectively. None of the metabolites, for which analyses were conducted, had quantifiable levels.

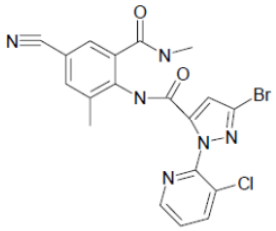
Under the conditions of this study in the dogs, cyantraniliprole produced robust treatment related effects at 1000 ppm and above. At 200 ppm, there were increases in liver weights accompanied by significant increase in alkaline phosphatase and decreases in albumin level. Therefore, the no-observed-adverse-effect level (NOAEL) was 40 ppm (1 mg/kg bw/day) for males and females. The LOAEL was 200 ppm (6 mg/kg bw/day) in males and females based on decreases in albumin levels, statistically significant increases in liver weight and alkaline phosphatase. At the next dose level (1000 ppm) histopathological changes characterized by hepatocellular degeneration and inflammatory process with associated increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT), and decreases in total protein and albumin were found.

This study is fully reliable (acceptable/guideline) and satisfies the data requirements for a chronic toxicity study in dogs (OPPTS 870.4100; OECD 452).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
3-Bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide (IUPAC name)



Lot/Batch #: HGW86-230
Purity: 94.5%
Description: Light white powder
CAS # 736994-63-1
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 14 days at room temperature or refrigerated (4°C), was distributed uniformly in the feed and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.

2. Vehicle and/or positive control: Untreated diet

3. Test animals
Species: Dog
Strain: Beagle
Age at dosing: Approximately 8.5–9 months old
Weight at dosing: 10.06–13.39 kg for males; 6.80–10.96 kg for females
Source: Covance Research Products, Inc., Kalamazoo, MI
Acclimation period: 28 days
Diet: Block Lab Diet® (Certified Canine Diet #5007, PMI Nutrition International, Inc.) was offered *ad libitum* upon arrival (Day -28). Meal Lab Diet® (Certified Canine Diet #5007, PMI Nutrition International, Inc.) was offered *via* approximately 400 g daily for approximately 2 hours beginning on Day -26. This regimen was followed daily through Week 45. Beginning Week 46 through the end of the study,

approximately 500 g was offered daily. During the test period, test substance was incorporated into the feed of all animals except negative controls.

Water: Tap water, *ad libitum*

Housing: Animals were housed singly in raised runs in an environmentally controlled room.

4. Environmental conditions

Temperature:	64–84°F
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

06-February-2008 to 04-February-2009

In-life initiated/completed

06-February-2008 to 04-February-2009

2. Animal assignment and treatment

Four groups of 4 animals/sex/concentration were administered cyantraniliprole in feed daily for 364 days at dietary concentrations of 0, 40, 200, and 1000 ppm (Table 1). One group of 7 animals/sex was administered diet at a concentration of 5000 ppm cyantraniliprole in feed daily for 12 weeks; at this point, four animals/sex (main study animals) continued to receive test diet, while the remaining surviving animals (recovery animals: 2 males, 3 females) were placed on control diet for the remainder of the study to evaluate reversibility of test article-related effects. The dietary concentrations were selected by the sponsor study monitor on the basis of the results of a 90-day dietary toxicity study in dogs (MRID 48119948) and other available data. Animals were assigned to dose groups by a standard block randomisation procedure using body weights as the block for females and testes volume as the block for males. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Groups, dogs/group, dietary concentration, and main daily compound intake.

Group no.	No./sex/ group	Conc. in diet (ppm)	Mean daily intakes[b] mg/kg bw	
			Males	Females
1	4	0 (control)	0	0
2	4	40	1	1
3	4	200	6	6
4	4	1000	27	27
5 [a]	7	5000	144 (124)[c]	133 (135) [c]

[a]: The 5000 ppm group consisted 7 dogs/sex, with 3 male and 3 females planned for the recovery study; the recovery animals received 5000 ppm for 12 weeks and then placed on the control diet for the remainder duration of the study (≈9 months).
[b]: Data excerpted from pages 185 & 187 of the report. The values were rounded to whole numbers.
[c]: Value in the parenthesis represents the compound intake for the recovery group with 12 weeks of treatment.

3. Diet preparation and analysis

The test substance was added to ground meal diet and thoroughly mixed for approximately 5 minutes. The resulting premix was added to additional meal diet and was blended for approximately 10 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. The stability, homogeneity, and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC (week 1 for stability and homogeneity; weeks 1, 12, 24, 36, and 48 for concentration). The test substance was at target concentrations ± 5.6% nominal, homogeneous (RSD range = 1 to 7%) throughout the feed and was stable (88.8 to 104.8% of nominal) for up to 14 days at room temperature or refrigerated. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics. The statistical methods used in this study are shown in Table 2.

Table 2. Statistics methods

Endpoints	Type of analysis
Body weights Weekly body weight Weekly body weight change Interval body weight change Food consumption Weekly food consumption Interval food consumption Hematology (except leukocyte counts) Clinical chemistry Organ weights Absolute weights Relative to body and brain weights	Group pair-wise comparisons (Levene's/ANOVA-Dunnett's/Welch's)
Leukocyte counts Total leukocyte counts Differential leukocyte counts	Log transformation/group pair-wise comparisons
Food efficiency Weekly food efficiency Interval food efficiency	Rank transformation with Dunnett's Test

Endpoints	Type of analysis
Urinalysis Urine volume pH Osmolality	

C. METHODS

1. Observations

Animals were observed twice a day for mortality, morbidity, injury, and the availability of food (once per day when the observation coincided with the daily feeding period) and water. Detailed examinations for clinical signs of toxicity were conducted twice daily. Neurobehavioral observations were conducted weekly. Neurobehavioral observations included changes in level of activity, gait, posture, altered strength, and response to handling as well as the presence of clonic or tonic movements, stereotypies (*e.g.*, excessive grooming, repetitive circling), or bizarre behaviour (*e.g.*, self-mutilation).

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded daily for each animal and group mean food consumption was calculated over the weekly weighing interval. Food efficiency and daily intake of the test article were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, urinalysis)

Clinical pathology evaluations were conducted on all main study animals (including animals at 5000 ppm designated for recovery) during Weeks -3, -2, 13, 26, and 52. All animals in the control and 5000 ppm recovery groups were also evaluated on Day 120 (Week 18) to evaluate recovery from any effects observed at the end of the 12-week exposure period. An additional blood sample was collected from one male at 5000 ppm on Days 65 and 80 and from one 5000 ppm female animal on Days 170 and 176 for clinical pathology evaluations (amylase and lipase), to evaluate clinical signs of toxicity in these animals. A blood sample was also collected on Day 176 for possible serum protein electrophoresis, but was not evaluated. Animals were fasted overnight prior to sample collection. Haematology, coagulation, clinical chemistry, and urine analysis were all preformed on the samples.

Hematology Parameters

Leukocyte Count Erythrocyte Count

Hemoglobin	Hematocrit
RBC Distribution Width	Platelets
Total Absolute Reticulocyte Count	% Reticulocyte Count
Differential Leukocyte Count	Blood Cell Morphology Count
Mean Corpuscular Volume (MCV)	Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin (MCHC)	

Coagulation Parameters
Activated Partial ThromboplastinTime (aPTT)
Prothrombin Time (PT)

Clinical Chemistry Parameters

Sodium	Potassium	Chloride
Calcium	Phosphorus	Alkaline Phosphatase (ALP)
Total Bilirubin		Gamma Glutamyltransferase (GGT)
Aspartate Aminotransferase (AST)		Alanine Aminotransferase (ALT)
Sorbitol Dehydrogenase (SDH)		Urea Nitrogen
Creatinine		Total Protein
Albumin		Globulin
Albumin/Globulin (A/G) Ratio		Cholesterol
Glucose		Triglyceride
Amylase		Lipase
Serum Protein Electrophoresis (SPE)		

Urinalysis Parameters

Color	Appearance
Volume	Specific Gravity
Microscopic Elements	pH
Protein	Glucose
Ketones	Bilirubin
Occult Blood	Urobilinogen
Osmolality	

6. Analysis of plasma concentration of test article and metabolites

   Blood samples (approximately 2 mL) were collected from all main study and recovery animals *via* the jugular vein for determination of the plasma concentrations of the test article and metabolites. Samples were collected 2 hours after food removal on Week 39. The animals were not fasted prior to blood collection. Plasma was prepared, frozen at approximately -70°C, and shipped to the Sponsor for analysis. Samples were evaluated by liquid chromatography-mass spectroscopy (LC-MS-MS).

7. Sacrifice and pathology

   All dogs were euthanized by anaesthesia with sodium pentobarbital and exsanguination. Gross examinations were performed on all main study animals. Organs that were

weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on protocol-designated sections of tissues (Table). All animals had a complete list of tissues collected. Based on the initial histological examination of animals at 0 and 5000 ppm, the arteries (coronary arteries [left anterior descending artery, left circumflex artery, right coronary artery], aorta, bracheocephalic trunk, left carotid artery, right carotid artery, left subclavian artery, right subclavian artery), cervical spinal meninges, esophagus, epididymides, urinary bladder, thymus, thyroid, stomach, heart, liver, and gall bladder were considered to be possible target organs and were examined for all animals at 40, 200, and 1000 ppm and all 5000 ppm recovery animals.

Table 3. Organs/tissues collected for pathological examination

Organ	Organs weighed	histopathologic evaluation conducted[c]
Adrenal (2)[d]	X	X
Aorta		X
Artery (left/right carotid/subclavian, lad, lcx, rca)[e]		X
Bone with marrow (femur)		X
Bone with marrow (rib)		X
Bone with marrow (sternum)		X
Bone marrow smear (2 collected)[a]		X
Bracheocephalic trunk		X
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X
Cervical spinal meninges		X
Epididymis (2)[d]	X	X
Eye (with retina) including optic nerve (2)[d]		X
Gallbladder[b]	X	X
Gastrointestinal tract:		
Esophagus		X
Stomach [cardia, fundus, and pylorus]		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Gonads:		
Ovary with oviduct (2)[d]	X	X
Testis (2)[d]	X	X
Gross lesions		X
Heart	X	X
Joint, tibofemoral		X
Kidney (2)[d]	X	X
Larynx		X
Liver (3 sections collected; 2 examined)[b]	X	X
Lung (collected whole; 2 sections examined)		X
Lymph nodes: mandibular, mesenteric, and tracheobronchia		X
Mammary gland (process females only)		X

Organ	Organs weighed	histopathologic evaluation conducted[c]
Nose levels (a, b, c, and d)		X
Nictitans gland (2)[d]		X
Pancreas		X
Peyer's patch		X
Pharynx		X
Pituitary		X
Prostate		X
Salivary gland, mandibular (2 collected; 1 examined)		X
Salivary gland, sublingual (2 collected; 1 examined)		X
Sciatic nerve		X
Skeletal muscle, biceps femoris		X
Skin		X
Spinal cord (cervical, thoracic, and lumbar)		X
Spleen	X	X
Thymus		X
Thyroid/parathyroid (2)[d]	X	X
Tongue		X
Trachea		X
Urinary bladder		X
Uterus (both horns)/cervix	X	X
Vagina		X

[a] Bone marrow smears were collected at the scheduled necropsy and held.

[b] Liver and gallbladder weighed together.

[c] Evaluated in control and high dose (5000 ppm) animals. Potential target tissues evaluated in all groups, including 5000 ppm recovery animals.

[d] (2) = Paired organ

[e] lad artery - left anterior descending artery
lcx artery - left circumflex artery
rca artery – right coronary artery

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

One male dog in the 5000 ppm main study group displayed clinical signs of decreased activity, ataxia, and convulsions, all of which were considered to be secondary to treatment-related arteritis. No additional treatment related clinical findings were observed.

2. Mortality

One male and one female animal at 5000 ppm were euthanized *in extremis* on Days 80 and 176, respectively. The need for euthanasia of the male animal was considered to be due to complications associated with arteritis. This death may be test article related as arteritis was observed in other dogs on this study in the 1000 and 5000 ppm groups. The finding of arteritis was also demonstrated at 10000 ppm in a 90-day study (MRID 48119948) and at 40000 ppm in a 28-day in dogs (MRID 48119942). The need to euthanize the female animal was not considered directly related to the test article as the

likely cause of the animal's poor condition appeared to be septicemia from an unknown cause.

3. Neurobehavioral observations

No treatment-related neurobehavioral findings were observed, except changes in body tone observed in the 5000 ppm male euthanized prior to scheduled sacrifice, and attributed to test article-related arteritis.

B. BODY WEIGHT AND BODY WEIGHT GAIN

The mean body weight in the 5000 ppm males was slightly reduced relative to the controls (↓6-7%) at various measuring intervals; however, the decrease showed no statistical significance (Table 4). The body weights of the females were comparable among all dose groups. The body weights of recovery group males and females were comparable to those of the controls.

The body weight gains of the 5000 ppm males and females were decrease relative to those of the controls during the intervals of 1-12 and 1-52 weeks (Table 5). At 1000 ppm, decreased body weight gain also seen in females during intervals of 1 to 52 weeks. Mean body weight gain in the 5000 ppm recovery males and females was comparable to control over the recovery interval, during which time body weight gain in main study animals was below that of the controls. Again the difference showed no statistical significance.

Table 4. Body weights (kg)

	Main Study					Recovery [a]
ppm	**0**	**40**	**200**	**1000**	**5000**	**5000**
Males						
Week 1	11.5±1.1	11.7±0.8	12.1±0.5	12.1±0.6	10.8±0.9 (↓6%)	12.0±1.9
Week 12	12.5±0.8	13.0±0.9	12.8±0.7	12.9±0.7	11.8±1.8 (↓6%)	13.3±1.8
Week 52	13.0±0.7	13.5±0.8	13.5±0.7	13.4±0.4	12.0±0.6(↓7%)	13.8±2.5
Females						
Week 1	9.3±0.9	9.1±1.1	8.9±0.8	8.5±1.0	9.1±0.8	9.7±1.0
Week 12	9.7±0.7	9.9±1.2	9.1±1.1	9.1±0.8	9.7±1.0	10.3±1.6
Week 52	10.6±0.9	10.6±1.8	9.8±1.0	9.3±0.8	9.6±0.9	11.2±2.8

[a] Two males and three females at 5000 ppm were placed on recovery following 12 weeks (84 days) of administration.
Data excerpted from pages 93-104 of the report.

Table 5. Body weight gain (kg)

	Main Study					Recovery [a]
ppm	0	40	200	1000	5000	5000
Males						
Week -1–12	1.2±0.5	1.6±0.3	1.4±0.5	1.1±0.3	0.9±0.4	1.2±0.1
Week -1–52	1.7±0.4	2.1±1.4	2.1±0.7	1.6±0.9	1.1±1.1	1.7±0,6
Females						
Week -1–12	0.7±0.5	1.3±0.8	0.6±0.4	0.7±0.3	0.5±0.4	1.2±0.5
Week -1–52	1.5±0.4	2.1±1.4	1.3±0.3	0.9±0.5	0.1±0.7	2.0±1.2

[a] Two males and three females at 5000 ppm were placed on recovery following 12 weeks (84 days) of administration.
Data excerpted from pages 119-130 of the report.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

Total food consumption mean values of treated animals were comparable to control, including mean food consumption during recovery (Table 6). Total mean feed efficiency demonstrated a similar pattern to that of body weight gain. Mean body weight gain and food efficiency in the recovery group were comparable to that of control over the recovery period.

Table 6. Food consumption, food efficiency, and compound intake in males and females.

	Main Study					Recovery[a]
ppm	0	40	200	1000	5000	5000
Males						
Food consumption (g/animal/day)						
Week 1-12	329.3±43.2	318.6±53.1	365.5±32.5	329.1±35.2	315.7±41.3	315.3±12.4
Week 1–52	328.4±41.4	312.6±49.9	367.9±47.9	344.8±40.9	322.6±43.0	—
Food efficiency (%)						
Week 1–12	4.4	6.0	4.5	4.0	3.2	4.5
Week 1–52	1.5	1.9	1.6	1.3	1.0	—
Compound Intake[b]						
mg/kg bw/day	0	0.96	5.67	27.04	143.79	124.48
Females						
Food consumption (g/animal/day)						
Week 1–12	258.5±34.0	283.0±44.7	274.5±19.9	247.0±16.9	253.5±36.2	267.3±53.7
Week 1–52	261.1±45.9	281.9±46.9	283.2±31.1	247.4±15.7	255.1±30.2	—
Food efficiency (%)						
Week 1–12	3.3	5.3	2.5	3.6	2.2	5.2
Week 1–52	1.6	2.0	1.2	1.0	0.1	—
Compound Intake[b]						
mg/kg bw/day	0	1.12	6.00	27.11	132.95	134.83

[a] Two males and three females at 5000 ppm were placed on recovery following 12 weeks (84 days) of treatment.
[b]: Data excerpted from pages 185-188 of the report.

D. OPHTHALMOLOGICAL EXAMINATIONS

No statistically significant or biologically significant changes in the incidences of ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

There were no adverse, test article-related differences in haematology parameters in male or female dogs.

3. Coagulation

There were no adverse, test article-related differences among coagulation parameters in either sex at any interval.

2. Clinical chemistry

Statistically significant increases in alkaline phosphatase (ALP) were found in all groups of male dogs at 13 weeks and the increase persisted until the end of the study for the main study males (ranging from ↑169% to ↑1100% relative to the controls). It should be noted that the increase in ALP at 1000 ppm or above in males exceeded the published control range (50-100 U/L) (Derelanko, 2000). In addition, the concurrent control ALP values for males appeared to be rather low. In females, statistically significant increases in ALP were observed in 1000 and 5000 ppm groups (ranging from ↑420% to ↑739%) at 13 weeks and the increase persisted until the end of the study. Increase in alanine aminotransferase (ALT), and decreases in albumin, and total protein were observed in male and female animals at dietary concentrations of 1000 and 5000 ppm. Gamma glutamyltransferase (GGT) was also increased in the 5000 ppm male and female groups (Table 6).

Other effects on clinical chemistry were observed at these and lower concentrations that were considered possibly test article related, but not adverse. These included mildly reduced cholesterol (≥1000 ppm males and females), and minimal/mild elevations in ALP, ALT, and reductions in total protein and albumin, at 200 and/or 40 ppm in males and/or females. These effects were considered likely test article related but might not be adverse as they were changes of a direction and/or magnitude not associated with adverse effects (cholesterol, total protein). The changes at 200 and 40 mg/kg/day might be considered as adaptive effects due to hepatic enzyme induction, and some of these values were reported to be within the laboratory's historical control range.

The values of the clinical chemistry parameters for recovery group males and females were comparable to those of the controls after the recovery animals were placed on the control diet. However, these data can only be interpreted as the possible reversibility of the effects which were induced by a short duration (12 weeks) of treatment and would not apply to the effects resulting from 1 year of exposure to cyantraniliprole.

Table 7. Selective clinical chemistry parameter

	Week	Main Study					Recovery[a]
		0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm	5000 ppm
Males							
Alkaline phosphatase U/L	13	26.8±6.8	70.0±9.9**	109.8±10.2**	174.3±51.0**	318.8±159.7**	354.5±24.8
	26	23.5±8.7	79.0±15.1**	125.0±25.2**	207.8±76.1	402.8±198.8	37.0±17.0
	52	17.3±4.6	71.5±17.2**	117.5±9.6**	209.5±105.7	401.0±171.9	28.5±13.4
GGT U/L	13	3.0±0.0	3.3±0.5	4.0±0.8	3.8±1.0	5.0±2.2	4.0±1.4
	26	2.5±0.6	3.0±0.8	3.8±1.0	4.0±1.4	4.5±1.3	2.5±0.7
	52	4.5±0.6	4.3±1.0	4.5±1.9	5.8±1.5	8.0±2.2	5.0±0.0
ALT U/L	13	32.3±12.7	31.0±2.9	42.0±18.0	43.0±8.9	67.0±32.7	46.5±9.2
	26	31.5±11.6	33.5±7.5	53.3±13.5	50.8±16.9	94.0±40.2	31.0±0.0
	52	33.0±11.3	36.0±5.7	59.5±27.5	95.8±50.5	112.5±39.2	32.0±0.0
Total Protein g/dL	13	6.3±0.4	6.0±0.3	5.8±0.2	5.4±0.2*	5.6±0.4	5.4±0.0
	26	6.1±0.5	5.9±0.1	5.5±0.2	5.2±0.1	5.4±0.6	6.0±0.4
	52	6.3±0.5	5.9±0.1	5.6±0.1	5.3±0.2	5.7±0.4	6.1±0.4
Albumin g/dL	13	3.2±0.2	2.8±0.2	2.6±0.2*	2.4±0.2**	2.3±0.2**	2.2±0.1
	26	3.2±0.2	2.8±0.2	2.6±0.2*	2.4±0.2**	2.2±0.3**	3.2±0.4
	52	3.2±0.1	2.9±0.1*	2.6±0.2*	2.4±0.1**	2.2±0.4**	3.1±0.4
Cholesterol mg/dL	13	155.3±23.8	187.8±27.0	162.8±42.0	102.8±15.5	90. 5±21.63*	128.0±18.4
	26	144.5±17.3	182.5±34.5	148.5±39.7	99.5±13.6*	97.5±32.4	158.5±38.9
	52	145.5±23.2	185.0±24.4	134.3±21.5	95.8±24.1	108.0±30.8	165.0±48.1
Females							
Alkaline phosphatase U/L	13	43.8±13.5	54.3±14.9	142.0±76.0	229.8±115.2*	369.0±134.2**	261.7±79.3*
	26	53.5±42.4	65.3±27.4	151.8±69.8	237.3±77.5	445.0±325.6**	43.3±14.0
	52	38.8±14.7	58.0±20.8	151.0±55.4	280.0±77.3*	591.3±254.7**	39.0±4.6
GGT[e] U/L	13	3.5±0.6	4.3±2.1	3.8±0.5	4.5±1.3	6.0±0.0*	4.7±1.5
	26	3.3±0.5	3.0±1.2	3.0±0.0	5.0±2.2	7.0±1.0**	2.3±0.6
	52	5.3±1.0	5.0±1.2	5.5±0.6	7.3±2.6	10.3±1.2**	4.3±1.5
ALT[e] U/L	13	26.8±3.0	25.3±3.2	28.8±7.9	41.5±18.1	66.8±29.9*	44.0±18.3
	26	21.0±3.8	26.3±3.6	26.0±6.6	58.8±41.3	117.0±58.4**	21.3±3.1
	52	22.3±3.4	25.5±3.1	26.5±7.1	83.5±74.5	109.0±50.1*	20.7±1.2
Total Protein g/dL	13	6.0±0.2	6.0±0.4	5.8±0.4	5.2±0.2**	5.2±0.2**	5.1±0.2
	26	5.8±0.3	5.90.4±	5.6±0.4	5.0±0.3**	4.9±0.1**	5.8±0.1
	52	6.0±0.2	6.2±0.3	5.6±0.4	5.2±0.2**	4.9±0.2**	6.1±0.3
Albumin	13	3.1±0.2	3.0±0.1	2.9±0.2	2.4±0.1**	2.4±0.2**	2.2±0.3**

Table 7. Selective clinical chemistry parameter

	Week	Main Study					Recovery[a]
		0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm	5000 ppm
g/dL	26	3.1±0.3	3.1±0.3	2.9±0.3	2.4±0.2**	2.2±0.0**	2.9±0.3
	52	3.2±0.2	3.1±0.2	2.9±0.2	2.4±0.2**	2.1±0.1**	2.9±0.2
Cholesterol mg/dL	13	169.8±43.3	158.0±16.0	152.8±12.7	131.5±31.7	115.5±39.8	99.3±2.1*
	26	200.5±31.4	151.8±15.4	149.5±17.9	118.5±32.7**	132.0±12.5	215.0±64.2
	52	249.8±109.2	163.0±13.1	138.5±10.9*	122.3±38.1*[d]	121.7±30.2*	265.7±60.1

Data excerpted from pages 38-39 and 224-250 of the report.

[a] Recovery group (individuals dosed through Week 12); statistics were not run on males of the recovery group due to 2 males only.

* Statistically significant ($p<0.05$) ** $P<0.01$

ALT = Alanine aminotransferase; GGT = Gamma glutamyltransferase

3. Urinalysis

There were no adverse, test article-related changes in urine parameters in male or female dogs.

F. PLASMA CONCENTRATION (SUPPLEMENTAL STUDY, MRID 48208427)

During week 39 of the one-year toxicity study, blood samples were collected and analyzed and quantified for parent compound and several possible metabolites. The results showed that, the average parent cyantraniliprole concentration in plasma for the 5000 ppm male and female animals was similar at 62 µg/mL and 56 µg/mL, respectively (Table 8). The average cyantraniliprole parent plasma concentrations for the 5000 ppm recovery male and female animals were 20 ng/mL and 11 ng/mL, respectively. The plasma from the single control female animal which was analyzed did not have any quantifiable level of cyantraniliprole. None of the possible metabolites, IN-JCZ38, IN-K7H19, IN-K5A77, IN-JSE76, IN-K5A78, IN-K5A79, IN-PLT97, and IN-NXX70, had quantifiable levels in any of the analyzed samples. The method reporting limit for all analytes was 5 ng/mL plasma.

Table 8. Summary of plasma concentrations for parent cyantrniliprole (DPX-HGW86) and possible metabolites. (Data excerpted from page 15 of the Supplemental Report, DuPont-19180. MRID 48208427)

	Average Plasma Concentration (ng/mL)								
Group	HGW86	JCZ38	K7H19	K5A77	J8E76	K5A78	K5A79	PLT97	NXX70
Group 1, Female (n=1)	<5.00[a]	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Group 5, Male (n=4)	62200	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Group 5, Female (n=3)	56500	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Group 5, Recovery, Male (n=2)	19.7	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Group 5, Recovery, Female (n=3)	10.8	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Group mean liver with gall bladder weights (absolute, relative to body weight, and relative to brain weight) were increased in a dose dependent manner for all male test article-treated groups and for females ≥200 ppm (Table 9). Group mean thyroid/parathyroid weights (absolute, relative to body weight, and relative to brain weight) were statistically significantly increased for males at 5000 ppm, but were not associated with any microscopic pathology; therefore, the increases were not considered adverse. Group mean spleen weights (absolute, relative to body weight, and relative to brain weight) were decreased for all male test article-treated groups; but the differences were not considered test article related or adverse as there was no dose-response and mean control spleen weight was high due to one dog with a large spleen. In addition similar effects were not seen in female dogs.

Table 9. Selective organ weights

	Main Study					Recovery
ppm	**0**	**40**	**200**	**1000**	**5000**	**5000**
Males (n=4)						
Liver w/gallbladder						
Absolute (g)	317.9±41.7	**411.0±40.1***	**449.9±27.9****	**492.9**±29.4**	**564.8**±73.1**	337.4±72.4
Relative to BWt[d] (%)	2.6	3.2	**3.5***	**3.9****	**5.0****	2.6
Relative to BrWt[d] (ratio)	3.9	4.7	**5.4***	**6.1****	**7.1****	4.2
Spleen						
Absolute (g)	95.3±22.1	73.2±7.1	61.3±25.6*	59.9±607*	66.5±14.1	59.2±20.4
Relative to BWt[d] (%)	0.772	0.575	0.484*	0.477*	0.586	0.5
Relative to BrWt[d] (ratio)	1.163	0.842	0.741*	0.746*	0.823	0.7
Thyroid/parathyroid						
Absolute (g)	1.0±0.3	1.1±0.2	1.2±0.2	1.3±0.1	**1.6±0.3***	1.1±0.6
Relative to BWt[d] (%)	0.009	0.009	0.010	0.010	**0.014****	0.0
Relative to BrWt[d] (ratio)	0.013	0.013	0.015	0.016	**0.020****	0.009
Females (n=4)						
Liver w/gallbladder						
Absolute (g)	299.5±22.7	285.3±41.5	**340.9±42.2**	**400.5±44.5**	**432.4±70.7***	298.2±89.5
Relative to BWt[d] (%)	3.003	2.924	**3.704***	**4.765***	**4.797****	2.8
Relative to BrWt[d] (ratio)	4.029	3.953	**4.431**	**5.284**	**5.829***	3.8
Spleen						
Absolute (g)	54.7±17.8	65.2±18.2	70.3±20.3	64.1±32.0	61.7±9.2	66.0±15.6
Relative to BWt[d] (%)	0.549	0.689	0.771	0.749	0.690	0.6

	Main Study					Recovery
ppm	**0**	**40**	**200**	**1000**	**5000**	**5000**
Relative to BrWt[d] (ratio)	0.730	0.895	0.908	0.856	0.833	0.8

* Significantly different control; ($p < 0.05$). Data excerpted from pages 46-49 and 267-285 of the report.

** Significantly different control; ($p < 0.01$)

[d] BWt = Body weight; BrWt = Brain weight

Bolded values were interpreted to be test-substance related increases, as compared to control values.

2. Gross pathology and histopathology

Test article-related macroscopic observations were observed in one female at 1000 ppm and one female at 5000 ppm and consisted of discoloration (tan) and/or irregular surface of the liver; these observations correlated microscopically with test article-related inflammation. There were no macroscopic observations at the recovery necropsy.

Test article-related microscopic findings that were considered adverse were present in arteries, liver, and gallbladder (Table 10). Increases in the incidence of arteritis were seen in 1000 ppm males and in 5000 ppm males and females in this study; similar finding was also seen in 28-day and 90-day oral toxicity dog studies (MRID 48119942, and MRID 48119948, respectively) at $\geq$ 10000 ppm. These results suggested the test article induced arteritis in dogs at dose levels $\geq$ 1000 ppm. The registrant presented literature references to show that the increases in the incidence of arteritis in cyantraniliprole treated dogs were "perhaps by exacerbating the incidence of spontaneous arteritis in beagle dogs" known as canine juvenile polyarteritis syndrome (CJPS). However, as discussed in the Data Evaluation Reports for 28-day and 90-day oral toxicity study in dogs, CJPS was thought to be mediated by the immune system, but cyantraniliprole did not show any effect on the immune system in two immunotoxicity studies in rats and mice (MRID 48119971 & MRID 48119972). It is difficult to differentiate CJPS from xenobiotic induced arteritis. Based on the currently available data, the increase in the incidence arteritis seen in this study at the 1000 and 5000 ppm in males and 5000 ppm in female was considered compound-related.

In the liver, both degenerative and inflammatory processes were observed in most males and females at 1000 ppm and 5000 ppm. In the centrilobular region, minimal to moderate hepatocellular degeneration was present and characterized by enlarged hepatocytes, rarefaction and margination of cytoplasm, vacuolation of cytoplasm, cytoplasmic membrane inclusions, and/or individual cell necrosis/lysis. In the portal regions, minimal to mild chronic-active inflammation was present and characterized by portal expansion with infiltrates of inflammatory cells and increased fibrous connective tissue, fibrous bridging between portal regions, pigment deposition, and/or bile duct hyperplasia.

Three males and one female at 5000 ppm had minimal to mild hyperplasia of the mucosa of the gallbladder. These effects were not seen in the recovery group males and females; **however, the finding in the recovery group should not be interpreted as to demonstrate that the effects seen in the 1-year treated dogs as reversible because**

the dogs in recovery group were treated with cyantraniliprole for only 12 weeks.

Table 10. Incidences of microscopic effects

	Main Study (52 week of dosing)					Recovery (12 wk of dosing)
ppm	**0**	**40**	**200**	**1000**	**5000**	**5000**
Males: n	**4**	**4**	**4**	**4**	**4**	**2**
Arteritis	1	0	0	**3**	**2**	0
Liver						
Degeneration, hepatocellular	0	0	0	**3**	**4**	0
Inflamation, chronic-active	0	0	0	0	**4**	0
Cholestasis	0	0	0	**1**	**3**	0
Gallbladder mucosa hyperplasia,	0	0	0	0	**3**	0
Females : n	**4**	**4**	**4**	**4**	**3**	**3**
Arteritis	0	0	0	0	**1**	0
Liver						
Degeneration, hepatocellular	0	0	0	**4**	**3**	0
Inflammation, chronic-active	0	0	0	**2**	**3**	0
Cholestasis	0	0	0	0	**1**	0
Gallbladder mucosa hyperplasia	0	0	0	0	**1**	0

Data excerpted from pages 52-54 and 287-351 of the report.

CONCLUSION

Under the conditions of this study, cyantraniliprole produced robust treatment related effects at 1000 ppm and above. At 200 ppm, there were increases in liver weights (absolute and relative) accompanied by significant increase in alkaline phosphatase and decreases in albumin level. Therefore, the no-observed-adverse-effect level (NOAEL) was 40 ppm (1 mg/kg bw/day) for males and females. The LOAEL was 200 ppm (6 mg/kg bw/day) in males and females based on decreases in albumin levels, statistically significant increases in liver weight and alkaline phosphatase. At the next dose level (1000 ppm) histopathological changes (hepatocellular degeneration and inflammatory process) with associated increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT), and decreases in total protein and albumin were observed.

The biochemical analysis on the blood sample showed that the only detectable analyte in plasma was parent cyantraniliprole, and there was no apparent sex difference in analyte concentration in any treatment groups. At the week 39, the plasma levels were 62 µg/mL and 57 µg/mL in the 5000 ppm males and females, respectively. None of the metabolites, for which analyses were

conducted, had quantifiable levels.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.4100; OECD 452) for a chronic oral toxicity in dogs.

Reference

Derelanko, MJ (2000). Toxicologist's Pocket Handbook. CRC Press LLC. pp 104-105.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.7.4/01

Report: Mukerji, P. (2009); DPX-HGW86 technical: Subchronic oral neurotoxicity study in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-19186. Feb. 5, 2009. MRID 48119966. Unpublished.

Guidelines: U.S. EPA OPPTS 870.6200 (1998)
OECD 424 (1997),
EC Guideline Directive 2004/73/EC Method B.43 (2004)
MAFF Japan Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 90-day neurotoxicity feeding study (MRID 48119966), male and female Crl:CD®(SD) rats (12 rats/sex/concentration) were fed cyantraniliprole technical (94.5%; HGW86-230) in the diet at concentrations of 0, 200, 2000, and 20000 ppm (males: 0, 11, 116, and 1195 mg/kg bw/day); females: 0, 14, 137, and 1404 mg/kg bw/day) for 92-93 days. The following parameters were evaluated: body weights, body weight gain, food consumption, food efficiency, clinical signs, and gross pathology. A neurobehavioral test battery consisting of motor activity and functional observational battery (FOB) assessments was conducted on 12 rats/sex/group prior to cyantraniliprole treatment (baseline) and during weeks 4, 8, and 13. On test days 92 and 93, 6 rats/sex/group were perfused *in situ* with fixative. The peripheral and central nervous systems and selected muscle tissues from control and high dose groups were prepared for histological evaluation.

Under the conditions of the study, there were no test substance-related effects on the following parameters: body weights, body weight gains, food consumption, food efficiency, clinical signs of toxicity, survival, neurobehavioral parameters (FOB and motor activity), or gross and microscopic morphology of the nervous system in either male or female rats of any dose groups.

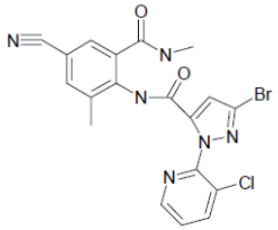
The no-observed-adverse-effect level (NOAEL) for neurotoxicity and systemic toxicity was 20000 ppm for male and female (1195 and 1404 mg/kg bw/day for males and females, respectively) (highest dose tested). No LOAEL could be established since the highest concentration did not produce any adverse test substance-related effects. The highest tested dose exceeded the limit dose (1000 mg/kg bw/day).

This study is classified as fully reliable (acceptable/guideline) and meets the guideline requirements of subchronic neurotoxicity study (U.S. EPA OPPTS 870.6200 and OECD 424).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Off-white
CAS #:	736994-63-1
Stability of test compound:	The test material in the diet was stable for at least 14 days at room temperature and 21 days refrigerated, was distributed uniformly in the feed, and was present in the feed at the target concentrations.

2. Vehicle and/or positive control: None

3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)
Age at dosing:	Approximately 6 to 8 weeks old
Weight at dosing:	187.8–254.3 g for males; 137.6–189.5 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	13 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   January 28, 2008 to February 02, 2009

2. Animal assignment and treatment

   Four groups of 12 animals/sex/concentration were administered cyantraniliprole in feed daily for 90 days at dietary concentrations of 0, 200, 2000, and 20000 ppm (Table 1). The 20000 ppm concentration was expected to provide a limit dose exposure (approximately 1000 mg/kg/day). Other concentrations were based on the results from a previous 90-day feeding study in rats (MRID 48119945); the study provided a NOAEL (100 ppm) and a LOAEL of 400 ppm based on thyroid follicular cell hypertrophy, increased thyroid weights and alterations in thyroid hormone homeostasis in female rats.

   Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study Design

Conc. In diet (ppm)[a]	No./sex group	Males	Females
		Mean daily intakes[b] mg/kg bw	Mean daily intakes[b] mg/kg bw
0	12	0	0
200	12	11	14
2000	12	116	137
20000	12	1195	1404

[a]: Weight/weight concentration of the test substance (adjusted for purity).
[b]: Data excerpted from pages 48-51 of the report.

3. Diet preparation and analysis

   The test substance was added to the rodent diet and thoroughly mixed for 6 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. The administered doses were adjusted for purity. The homogeneity and concentration of cyantraniliprole in the dietary mixtures were evaluated using HPLC and UV analysis near the beginning of the study. In addition, concentration was evaluated near the middle and end of the study. The stability of the cyantraniliprole in the dietary mixtures was confirmed in another study being conducted concurrently. The test substance was at target concentrations (±9.0%), homogeneous (90.5 to 99.5%) throughout the feed, and was stable (based on results from a concurrent 2-year rat study) for up to 14 days at room temperature, and 21 days refrigerated.

4. Statistics: Statistical methods used in this study are presented in Table 2. The methods appeared to be adequate for analyzing the results of this study.

Table 2. Statistics

Parameter	Preliminary test	Method of statistical analysis	
		If preliminary test is not significant	**If preliminary test is significant**
Body weight Body weight gain Food consumption Food efficiency	Levene's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Motor activity[b] Grip strength Foot splay Body temperature Rearing	Levene's test for homogeneity and Shapiro-Wilk test for normality[c]	Repeated measures analysis of variance followed by Linear contrasts	Sequential application of the Jonckheere-Terpstra trend test
Incidence of FOB Descriptive parameters	None	Cochran-Armitage test for trend [d]	

[a] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[b] Test day and 10-minute interval were used as repeated-measure factors.

[c] Normalizing or variance stabilizing transformation of the data was used if necessary.

[d] If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni-Holm adjustment was used.

C. METHODS

1. Observations

   Animals were observed at least once daily for mortality and morbidity and examined weekly for clinical signs of toxicity.

2. Body weights

   All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Functional observational battery and motor activity

   Functional Observational Battery (FOB) and Motor Activity (MA) assessments were conducted on 12 rats/sex/group prior to exposure (baseline), and during weeks 4, 8, and 13. The experimenter conducting the FOB was blind with respect to the treatment group of each animal. The parameters evaluated in the FOB are presented below.

Inside the home cage:

Posture
Palpebral closure
Gait/coordination abnormalities
Tremors
Convulsions

During removal from the home cage and handling:

Ease of removal Palpebral closure
Ease of handling Exophthalmus
Vocalizations Lacrimation
Muscle tone Salivation
Piloerection Dehydration
Fur/skin appearance Emaciation
Mucous membranes

In the open field arena:

Posture Arousal
Gait/Coordination Vocalizations
Tremors Diarrhea
Convulsions Polyuria
Muscle spasms/fasciculation Righting Reflex
Respiration Ease Palpebral Closure
Respiration Rate
The number of rearing movements

While in the standard arena:

Approach/touch
Auditory stimulus
Tail pinch

The remainder of FOB testing involved standardized or calibrated devices:

Fore- and hindlimb grip strength
Hindlimb splay
Rectal body temperature
Pupillary constriction with a beam of light.
Presence of diarrhea and polyuria on the cageboards

Motor Activity (MA):

Motor activity sessions were conducted on the same animals, the same day as FOB assessments, following the FOB assessments. Rats were individually tested in one of 30 nominally identical, automated activity monitors (Coulbourn®). Duration of movement and number of movements were evaluated in 6 consecutive blocks of 10 minutes each, as well as for the total 60-minute session.

5 Sacrifice and neuropathology

At termination, 6 animals per group were anesthetized and underwent whole body *in situ* perfusion. Gross examinations were performed on all study animals. The following tissue samples taken from the organs and tissues were saved from all test groups:

Brain
Forebrain
Cerebrum
Midbrain
Cerebellum
Pons
Medulla
Spinal cord (cervical and lumbar regions)
Cervical and lumbar dorsal root ganglia
Dorsal and ventral root fibers
Sciatic nerve, tibial nerve, sural nerve,
Eye (with optic nerve)
Gastrocnemius muscle

Tissues samples from rats in the control and high-dose groups were processed and evaluated microscopically.

6 Positive control data

Procedures and data describing the effects of the positive controls (trimethyltin, acrylamide, carbaryl, d-amphetamine, and scopolamine) are presented in 6 separate reports (References: 1, 2, 3, 4, 5,& 6).

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

Test substance-related mortality did not occur during the course of this study. However, one 2000 ppm male and one 20000 ppm male were sacrificed *in extremis* for humane reasons due to injury or due to location of an incidental mass, respectively.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains.

Table 3. Body weights and body weight gains (gm)

Study Day	Dose (mg/kg bw/day)			
	0	200	2000	20000
	Body weight (Males) (n=12)			
Day 0	229.9±11.3	229.7±15.7	228.7±10.4	232.7±111.8
Day 7	289.7±15.8	293.4±19.8	287.0±12.6	294.3±15.3
Day 49	480.7±39.8	492.1±41.6	477.4±17.6	476.7±37.7
Day 91	562.7±43.3	574.5±46.8	559.9±28.9	561.7±48.2
	Body weight gain (Males)			
Day 0-7	59.8±7.2	63.7±7.7	58.3±5.7	61.6±5.6
Day 49-56	14.2±6.6	12.2±8.6	10.9±4.7	12.9±5.5
Day 0-91	332.8±38.2	344.8±37.1	330.7±28.2	328.1±40.5
	Body weight (Females) (n=12)			
Day 0	163.1±10.0	161.4±11.5	164.6±11.3	164.6±7.0
Day 7	193.2±14.6	192.1±13.2	189.8±12.8	193.4±8.5
Day 49	275.5±21.3	274.2±.8	268.5±17.6	274.1±19.2
Day 91	303.1±29.5	300.7±24.7	292.5±23.4	300.5±20.7
	Body weight gain (Females) (n=12)			
Day 0-7	30.2±6.1	30.8±5.6	25.3±4.8	28.9±4.9
Day 49-56	1.1±7.1	2.1±8.6	3.1±4.5	0.2±9.1
Day 0-91	140.0±22.7	139.3±20.6	127.9±15.5	135.9±16.3

Data excerpted from pages 32-39 of the study report.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no compound-related changes on food consumption or food efficiency in any treated groups.

Table 4. Food consumption (g/animal/day)

Study Day	Dose (mg/kg bw/day)			
	0	200	2000	20000
	Males			
Day 0-7	22.9±1.7	23.3±2.0	22.3±1.0	23.4±1.4
Day 7-14	25.2±2.0	25.3±2.3	24.9±1.4	26.3±2.0
Day0-91	25.6±2.0	25.7±2.0	25.4±1.1	26.6±1.7
	Females			

Day 0-7	16.6±1.8	16.1±1.0	**14.9*±0.8**	15.6±0.6
Day 7-14	18.1±1.6	18.2±1.3	17.8±1.2	18.8±1.4
Day0-91	17.8±1.4	18.1±1.4	17.3±1.1	18.1±1.0

Data taken from pages 40-41, 44-45 *p<0.05

N= 12 or 11for day 0-91 for 2000 and 20000 ppm male groups (1 death in each of the 2000 and 20000 ppm male group)

D. FUNCTIONAL OBSERVATIONAL BATTERY AND MOTOR ACTIVITY

There were no test substance-related effects in either males or females on forelimb or hindlimb grip strength, foot splay, rearing or any of the other parameters of the FOB. In addition, there were no test substance-related changes on motor activity (duration or number of movements) in any test compound- treated groups relative to the controls (Table 3 and 4).

Table 5.

Motor Activity Assessment: Mean Duration of Movement (Sec) for Male Rats

GROUP	CONC. (ppm)	N	SUCCESSIVE 10-MINUTE INTERVALS 1	2	3	4	5	6	TOTAL
BASELINE									
1	0	12	364 (39)	241 (59)	175 (90)	63 (89)	26 (59)	4 (7)	873 (247)
2	200	12	378 (50)	263 (73)	144 (86)	69 (96)	44 (66)	7 (20)	905 (320)
3	2000	12	381 (47)	258 (57)	166 (86)	77 (80)	36 (89)	15 (31)	932 (247)
4	20000	12	379 (63)	293 (55)	205 (77)	103 (80)	39 (64)	21 (41)	1039 (265)
WEEK 4									
1	0	12	384 (54)	313 (48)	239 (102)	199 (131)	154 (106)	162 (138)	1452 (455)
2	200	12	384 (53)	328 (66)	274 (80)	223 (91)	180 (81)	153 (133)	1542 (413)
3	2000	12	388 (45)	328 (67)	289 (70)	231 (78)	214 (60)	146 (104)	1596 (277)
4	20000	12	378 (57)	303 (42)	237 (63)	194 (73)	150 (105)	125 (104)	1386 (326)
WEEK 8									
1	0	12	396 (44)	283 (94)	254 (86)	178 (109)	176 (87)	147 (128)	1434 (472)
2	200	12	369 (61)	299 (90)	210 (107)	189 (101)	169 (92)	129 (121)	1364 (469)
3	2000	11	374 (43)	302 (71)	231 (64)	180 (77)	177 (113)	164 (101)	1427 (273)
4	20000	12	378 (61)	286 (79)	235 (93)	220 (74)	187 (62)	193 (108)	1500 (353)
WEEK 13									
1	0	12	370 (52)	289 (75)	195 (93)	187 (62)	159 (84)	155 (123)	1354 (411)
2	200	12	359 (52)	296 (67)	208 (103)	207 (107)	188 (101)	157 (86)	1415 (436)
3	2000	11	366 (51)	291 (61)	246 (78)	190 (66)	182 (72)	176 (88)	1451 (311)
4	20000	11	375 (68)	288 (68)	214 (59)	198 (64)	188 (94)	151 (70)	1415 (307)

Data arranged as: Mean (Standard Deviation).
N = Number examined.

There were no statistically significant differences compared to the control group at p < 0.05.

Table 6.

Motor Activity Assessment: Mean Duration of Movement (Sec) for Female Rats

GROUP	CONC. (ppm)	N	SUCCESSIVE 10-MINUTE INTERVALS 1	2	3	4	5	6	TOTAL
BASELINE									
1	0	12	338 (45)	210 (63)	158 (99)	113 (120)	48 (76)	20 (63)	886 (309)
2	200	12	345 (48)	183 (83)	136 (81)	97 (93)	50 (58)	30 (43)	840 (197)
3	2000	12	357 (61)	238 (93)	153 (96)	101 (111)	82 (96)	9 (10)	940 (329)
4	20000	12	365 (42)	231 (80)	153 (83)	109 (112)	23 (31)	33 (74)	913 (247)
WEEK 4									
1	0	12	359 (67)	281 (83)	236 (88)	179 (100)	135 (82)	98 (102)	1287 (369)
2	200	12	350 (53)	250 (70)	204 (84)	141 (74)	107 (80)	74 (79)	1126 (186)
3	2000	12	375 (70)	281 (84)	218 (94)	140 (89)	142 (87)	120 (92)	1276 (399)
4	20000	12	376 (32)	250 (84)	224 (108)	177 (88)	145 (92)	118 (102)	1291 (320)
WEEK 8									
1	0	12	365 (46)	265 (50)	210 (81)	177 (44)	146 (83)	92 (79)	1255 (209)
2	200	12	368 (55)	208 (71)	159 (70)	175 (85)	156 (80)	149 (71)	1215 (269)
3	2000	12	386 (82)	232 (101)	204 (134)	209 (110)	213 (108)	170 (111)	1414 (517)
4	20000	12	395 (33)	249 (79)	207 (74)	194 (96)	170 (88)	173 (77)	1388 (336)
WEEK 13									
1	0	12	348 (61)	232 (49)	149 (83)	159 (82)	145 (67)	138 (67)	1171 (281)
2	200	12	366 (61)	195 (79)	159 (89)	149 (75)	130 (77)	139 (93)	1137 (331)
3	2000	12	356 (77)	237 (92)	171 (95)	166 (102)	161 (113)	189 (127)	1281 (518)
4	20000	12	378 (47)	242 (46)	173 (47)	187 (44)	166 (65)	131 (90)	1277 (201)

Data arranged as: Mean (Standard Deviation).
N = Number examined.

Tables 3 and 4 are excerpted from pages 68 and 69 of the study report.

E. NEUROPATHOLOGY

1 Gross pathology

No test substance-related gross lesions were observed at necropsy.

2. Neuropathology

Neuropathological evaluation of brain, spinal cord, and peripheral nerves did not reveal any test substance-related histological changes. However, one 20000 ppm males presented a small, solitary aggregation of mixed inflammatory cells in the dorso-lateral medulla. The lesion was considered to be an incidental inflammatory focus unrelated to treatment.

III. CONCLUSION

Under the conditions of the study, there were no test substance-related effects the following parameters: body weights, body weight gains, food consumption, food efficiency, clinical signs of toxicity, survival, neurobehavioral parameters (function observation battery and motor activity), or gross and microscopic morphology of the nervous system in either male or female rats of any dose groups.

The no-observed-adverse-effect level (NOAEL) for neurotoxicity and systemic toxicity was 20000 ppm (1195 and 1404 mg/kg bw/day for males and females, respectively) (highest dose tested). No LOAEL could be established since the highest concentration did not produce any adverse test substance-related effects.

This study is classified as fully reliable (acceptable/guideline) and meets the guideline requirements of subchronic neurotoxicity study (U.S. EPA OPPTS 870.6200 and OECD 424).

References:

1. DuPont Haskell (1995). Neurotoxicity Evaluation of Trimethyltin in Rats (Positive Control Study). Unpublished data, HLR 266-95.

2. DuPont Haskell (1996). Neurotoxicity Evaluation of Acrylamide in Rats (Positive Control Study). Unpublished data, HLR 293-95.

3. DuPont Haskell (1997). Neurotoxicity Evaluation of Carbaryl in Rats (Positive Control Study). Unpublished data, HL-1997-00361.

4. DuPont Haskell (1997). Neurotoxicity Evaluation of Amphetamine in Rats (Positive Control Study). Unpublished data, HL-1997-00686.

5. DuPont Haskell (2000). Neurotoxicity Evaluation of Carbaryl in Rats (Positive Control Study). Unpublished data, DuPont-3468.

6. DuPont Haskell (2002). Neurotoxicity Evaluation of Carbaryl and Scopolamine in Rats (Positive Control Study). Unpublished data, DuPont-7378.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.6.1 Two-generation reproductive study in the rat

Report: IIA 5.6.1/01 Barnett, J.F., Jr. (2011); DPX-HGW86 technical: Oral (diet) two-generation (one litter per generation) reproduction toxicity study in rats. Charles River Laboratories, Horsham, Pennsylvania, U.S.A. Report No.: AUV00033. DuPont-19187. April 21, 2011. MRID 48119967.
Unpublished.

Guidelines: OECD No. 416 (2001)

OPPTS 870.3800 (1998)
MAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a two-generation reproduction study (MRID 48119967), groups of Crl:CD®(SD) rats (30/sex/group) were fed cyantraniliprole (94.5%; HGW86-230) at concentrations of 0, 20, 200, 2000, and 20000 ppm (based on premating compound intake: 0, 1.4, 14, 136, and 1344 mg/kg bw/day for P_1 females; 0, 1.1, 11.0, 110, and 1125 mg/kg bw/day for P_1 males) for both the P_1 and F_1 generations). The P_1 generation rats received the test diet for at least 70 days before mating and continued until sacrificed. The F_1 generation rats received the test diet continuously as their respective P_1 generation sires and dams starting at weanling and continued until sacrifice. The F_1 rats were bred within their respective treatment groups to produce F_2 litters at least 86 days after weaning. The following parameters were evaluated: body weight, body weight gain, food consumption, food efficiency, clinical signs, reproductive indices, litter and pup parameters, oestrus cycle, sperm parameters, organ weights, and gross and microscopic pathology.

Under the conditions of the study, cyantraniliprole produced treatment-related effects on the parental animals at dietary concentrations of 200, 2000 and 20000 ppm. **For the parental animals**, reductions in body weight and body weight gains at 20000 ppm P_1 and F_1 generation male and female rats were found (absolute body weight:↓4% - ↓11%; body weight gains: ↓5% - ↓20%). A significant increase in the fixed thyroid lobes/parathyroid weights (absolute and relative) was seen in the 20000 ppm P_1 and F_1 males and in 200, 2000, and 2000 ppm P_1 and F_1 females. A corresponding dose-related increase in the incidence of the thyroid follicular epithelial cell hypertrophy/hyperplasia was found in the 2000 and 20000 ppm P_1 males and females and in 200, 2000, and 20000 ppm F_1 males and females. In addition, significant reductions in thymus weight accompanied by thymus atrophy were noted in 2000 and 20000 ppm P_1 female rats (thymus weight: ↓22% - ↓28%). Thymus weight in 20000 ppm F_1 females was also decreased (↓35%).

No treatment-related **reproductive effects** were found. The following parameter were

comparable across the test groups: estrous cyclicity, sperm analyses, mating behavior, conception and fertility, parturition, gestation length, lactation, weaning and the growth and development of offspring including onset of puberty.

For the offsprings, treatment-related effects included decreased body weights at 20000 ppm in the F_1 generation pups on PNDs 15 and 22 (↓11% - ↓14%), and in F_2 generation pups from birth to PND 22 (↓8% - ↓15%.) at 2000 and 20000 ppm. Pup thymus and spleen weights were deceased in 20000 ppm F_1 males and in 2000 and 20000 ppm F_2 males and females (↓19% - ↓26%). Decreases in 20000 ppm F_1 and F_2 pup brain weight and F_2 pup adrenal weight were also found. An increase in the number of 20000 ppm F_1 pups with dehydration was also seen.

The **parental NOAEL** was 20 ppm (1.4 mg/kg/day). LOAEL was 200 ppm (14 mg/kg/day) based on thyroid weight increase and corresponding dose-related increase in the incidence of thyroid follicular epithelial cell hypertrophy/hyperplasia. It should be noted that the data on ≥ 2000 ppm test animals were more robust in terms of number and severity of effects in demonstrating the toxicity of cyantraniliprole.

The **reproductive and fertility NOAEL** was 20000 ppm (1344 mg/kg/day) (the highest dietary concentration tested and greater than the limit dose for a reproduction study) based on the lack of adverse test substance-related effects on fertility and reproductive parameters at any dose levels in the study.

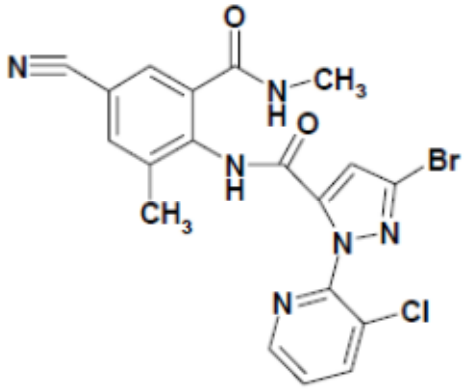
The **offspring** NOAEL was 200 ppm (14 mg/kg/day). LOAEL was 2000 ppm (136 mg/kg/day) based on dose-related decreases in organ weights (thymus and spleen) and pup body weight decrease in F_2.generation.

This study is classified as fully reliable (acceptable/guideline) and satisfied the guideline requirements (OPPTS 870.3800; OECD N0. 416; MAFF 12 Nousan 8147) for a reproduction and fertility study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
Synonym: 3-bromo-N-[-4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide.



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Solid white powder
CAS #:	736994-63-1
Stability of test compound:	The test material was reported to be stable in feed for at least 14 days at room temperature, was distributed uniformly in the feed and was present in the feed at targeted concentrations. Batches were prepared at approximately 2 week intervals.

2. Vehicle and/or positive control: Untreated diet

3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)
Age at dosing:	P1 generation approximately 6 weeks
Weight at dosing:	330–402 g for males; 204–239 g for females
Source:	Charles River Laboratories, Inc., - Males : (1) Quebec, Canada and (2) Raleigh, NC , USA; Females : (1) Portage, MI and (2) Raleigh, NC, USA.
Acclimation period:	4–6 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.

Water: Tap water passed through reverse osmosis membrane, *ad libitum*

Housing: Animals were housed singly during non-mating and gestation periods in stainless steel, wire-mesh cages suspended above cage boards. During cohabitation periods, rats were housed as breeding pairs. Beginning no later than gestation day 20, females were housed singly in nesting boxes until delivery. After delivery, females were housed with their litters in nesting boxes.

4. Environmental conditions

Temperature: 19–25°C
Humidity: 30–70%
Air changes: Minimum 10 exchanges/hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiation/completion

April 07, 2008 to February 01, 2011

2. Animal assignment and treatment

Animals were assigned to dose groups by computerized, stratified randomization. The experimental design is described in the Tables 1, 2, and 3. Animal housing and husbandry were carried out in accordance with the provisions in the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Treatment Groups and Dietary Concentrations

Dosage group	P_1 Generation number of rats per sex	F_1 Generation number of rats per sex	Dietary concentration (ppm)[a]
I	30	30	0
II	30	30 (M), 29 (F)	20
III	30	30	200
IV	30	30	2000
V	30	30[b]	20000

M – Male F – Female

[a] Weight/weight concentration of DPX-HGW86

[b] Rat 7574 was sacrificed on day 23 of study due to adverse clinical observations and was replaced by rat 7674 and excluded from study.

Data excerpted from page 43 of the report.

Table 2. Treatment schedule			
Generation	**Approximate age at start of feeding (days)**	**Approximate number of study days before mating**	**Duration of feeding**
P_1	42	70	Until sacrifice
F_1	21	86	Until sacrifice

Table 3. Sacrifice schedule		
Animals	**Generation**	**Schedule**
Adult males	P_1, F_1	After siring litters P_1 – test Days 110–113 After siring litters F_1 – test Days 102–116
Pregnant females	P_1, F_1	On day of weaning litters – postpartum Day 21
Nonpregnant females	P_1, F_1	Approximately Day 28 after the end of cohabitation
Day 4 culled pups	F_1, F_2	Day 4 of lactation
Weanlings	F_1, F_2	On day of weaning – postnatal Day 21 (except F_1 rats selected for parental rats)

3. Dose Selection

The report indicated that the dietary concentrations up to and including 20000 ppm (limit dose) were similar to those used in the rat carcinogenicity study (MRID 48122577). The rationale for the concentration selection for the rat carcinogenicity study was reported to be based on the results of the previous studies, which were not specified.

4. Diet preparation and analysis

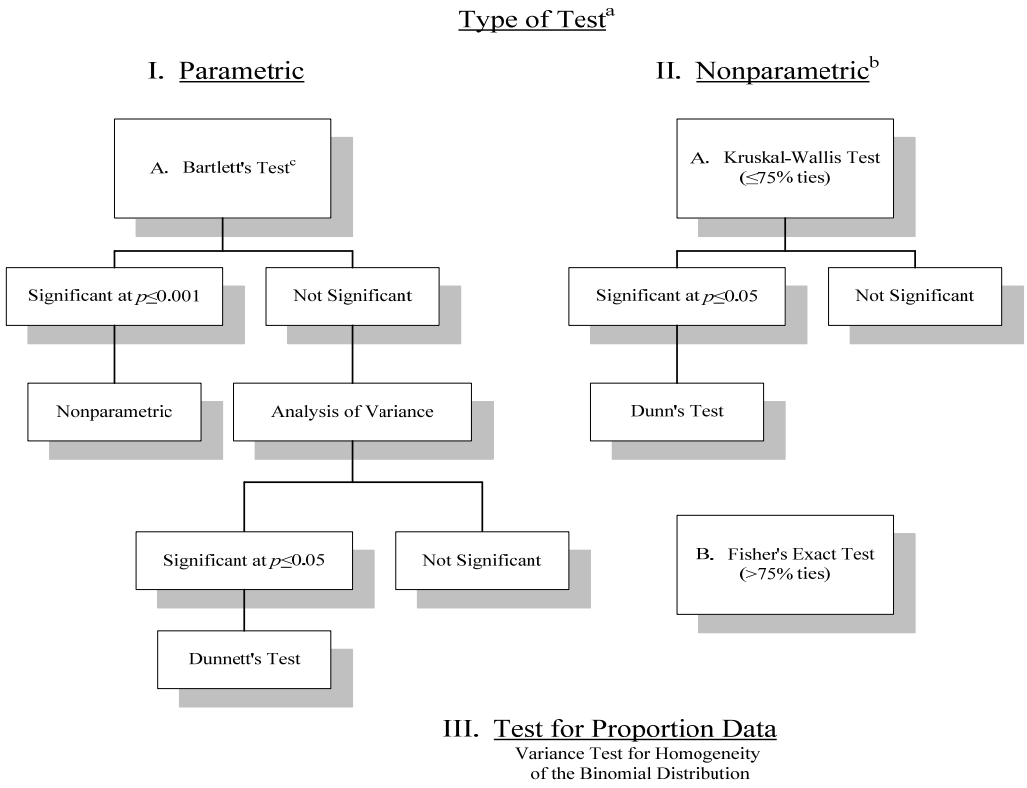
The test diets were prepared as needed, stored at room temperature and used within 30 days from the date of preparation. The report stated that diet samples were extracted with acetonitrile and test substance stability samples were dissolved in acetonitrile. Concentrations of cyantraniliprole in diet sample extracts and test substance solutions were measured by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The stability analyses conducted near the beginning and end of the study show that the test substance was stable during the study period. The data for diet samples prepared throughout the study show that the test substance was homogeneously mixed (RSD range = 1-8%), at the targeted concentrations (± 12.5% nominal), and stable for up to at least 14 days at room temperature and 21 days under refrigeration. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

The statistical analytic methods and schemes described in the report were judged to be appropriate and reproduced in this section. Data generated during the course of this

study were recorded either by hand or using the Argus Reproductive and Developmental Toxicology Data Collection System, the Hamilton Thorne IVOS and the Vivarium Temperature and Relative Humidity Monitoring System. All data were tabulated, summarized and/or statistically analyzed using the Argus Reproductive and Developmental Toxicology Data Collection System and/or the Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel (part of Microsoft® Office 97/2000/2003/XP), Quattro Pro 8 and/or The SAS System (version 6.12).

Averages and percentages were calculated. Litter values were used where appropriate. The following schematic, which is excerpted from page 53 of the report, represents the statistical analyses of the data:



a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Proportion data are not included in this category.
c. Test for homogeneity of variance.

Clinical observations and other proportional data were analyzed, using the Variance Test for Homogeneity of the Binomial Distribution.

Continuous data (*e.g.*, maternal body weights, body weight changes, feed consumption values, organ weights and litter averages for percent male pups, percent resorbed conceptuses, pup body weights and pup anomaly data) were analyzed, using Bartlett's

Test of Homogeneity of Variances and the Analysis of Variance, when appropriate [*i.e.*, Bartlett's Test was not significant ($p>0.001$)]. If the Analysis of Variance was significant ($p\leq0.05$), Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [*i.e.*, Bartlett's Test was significant ($p \leq0.001$)], the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present. In cases where the Kruskal-Wallis Test was statistically significant ($p \leq0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data.

Count data were evaluated, using the procedures described above for the Kruskal-Wallis Test.

C. METHOD

This experimental Method section as presented in the Tier II report by the registrant is consistent with the study report and is reproduced in the following paragraphs.

A two-generation reproduction study, involving the production of one set of litters in each generation, was conducted with cyantraniliprole. Crl:CD®(SD)IGS BR rats (30/sex/concentration) were fed diets containing 0, 20, 200, 2000, and 20000 ppm DPX-HGW86. Following at least 70 days of diet administration (premating), the P_1 and F_1 generation males and females were cohabitated within their respective treatment groups, to produce F_1 and F_2 litters, respectively. Vaginal smears were collected daily from all females during mating until evidence of copulation was observed. Dams were allowed to deliver and rear their offspring until weaning (postpartum Day 21). At weaning, 30 male and 30 female of F_1 rats/group except for the 20 ppm group (29 females) were randomly selected to comprise the F_1 generation and were given the same concentration level as their respective P_1 generation sires and dams. F_1 and F_2 litters were culled to 4 pups/sex/litter (litter size permitting) on postnatal Day 5; all remaining pups were sacrificed without further evaluation.

Clinical observations, and body weight and food consumption were determined weekly throughout the study. Litter examinations (live, dead or missing pups, individual pup weights, clinical observations) were determined at birth (Day 1), Day 5 and weekly during the lactation period. Oestrous cycle parameters (percent days in diestrus, proestrus, and estrus) and oestrous cycle length were evaluated for 3 weeks prior to cohabitation in P_1 and F_1 rats. The age at either vaginal opening or preputial separation was recorded for the F_1 generation. For P_1 and F_1 rats, sperm motility was determined from a sample from the vas deferens; sperm morphology and concentration were determined from a sample from the cauda epididymis.

Pups dead at Day 1 were examined to determine if they were stillborn pups or liveborn pups that died shortly after birth. All pups culled on Day 5 were sacrificed and examined for

gross lesions. Pups that died or were sacrificed before scheduled termination were examined for gross lesions and cause of death. F_1 weanlings not selected to comprise the F_1 generation and all F_2 weanlings were given a gross post-mortem examination and gross lesions were retained. The spleen, thymus, thyroid, adrenals and brain were weighed and retained from one weanling/sex/litter from F_1 and F_2 litters. After litter production, all P_1 and F_1 rats were given a gross pathological examination and the testes, epididymides, seminal vesicles (with coagulating gland), prostate, ovaries, uterus (with oviducts and cervix), brain, spleen, adrenal glands, pituitary, thyroid lobes and parathyroids (fixed weight), liver, kidney were weighed. On day 22 postpartum, brain, spleen, thyroid, adrenals and thymus were weighed and retained from one F_1 and F_2 generation pup/sex/litter.

Tissues and gross lesions from all P_1 and F_1 rats and target organs from F_1 weanlings in the control and 20000 ppm groups were examined microscopically; tissues in the low and intermediate groups were subsequently evaluated as needed to determine a no-adverse-effect level. Reproductive organs from the low and intermediate group P_1 and F_1 rats with suspected reduced fertility were examined microscopically. Ovarian follicle numbers were evaluated in control and 20000 ppm F_1 rats (10/sex/group).

II. RESULTS AND DISCUSSION

A. PARENTAL TOXICITY (P_1 and F_1 rats)

1. Clinical observations

   No treatment-related increase in death was found. Relative to the controls, a transient increase in the number of observation/number of animal (8/3) with urine-stained fur was seen in 20000 ppm P_1 and F_1 male rats in the early period of the study; no urine-stained fur was seen in the controls. This increase was not considered adverse due to transient nature of the finding and similar effect was not seen in females.

2. Body weight and body weight gain

   For P_1 males, body weight was slightly decreased in 20000 ppm (↓4-5% relative to the controls) (Table 4), and body weight gains were also reduced (10%) in the 20000 ppm for days 1 to termination of the study. The P_1 females also showed slight decrease in body weight (↓3-5%) relative to the controls during premating, gestation, and lactation periods. Similarly, there was a decrease in body weight gains in 20000 ppm P_1 females at various intervals (premating & gestation). Although these reduction did not demonstrate a statistically significance, they appeared to be treatment-related.

   The F_1 males showed statistically significant reduction in body weights during premating (≈↓10%) or before sacrifice in 20000 ppm group, and the body weight gains were also significantly decreased. In the 20000 ppm F_1 females, body weight was slightly reduced at all intervals during premating and gestation (↓6%).

Table 4. Summary of body weight and body weight gain data on P_1 and F1 parental male and female rats

Parameter	0 ppm	20 ppm	200 ppm	2000 ppm	20000 ppm
P_1 males					
Body weight (g)					
Body weight at end of premating (g)	600±44	605±52	599±72	588±47	576±49(↓4%)
Day 92 body weight (g)	630±44	641±61	622±79	615±52	601±49(↓5%)
Final body weight (g)	650±48	660±64	645±79	637±55	618±54(↓5%)
Body weight gain, WG (g)					
Day 1-70[a]	235±39	241±43	237±61	227±40	217±39 (↓8%)
Day 1-92	265±40	277±52	261±68	254±44	239±43(↓10%)
Day 1-termination	286±44	296±55	283±69	276±47	256±47(↓10%)
P_1 Females					
Body weight (g)					
Body weight at end of premating (g)	292±22	287±21	288±26	284±24	277±30 (↓5%)
Body weight at start of gestation (g)	291±18	291±25	288±24	285±20	281±31(↓3%)
Body weight at end of gestation (g)	446±29	442±26	410±33	431±28	428±45(↓4%)
Body weight at start of lactation (g)	329±21	329±24	320±24	318±24	315±31(↓4%)
Body weight at end of lactation (g)	353±25	349±25	341±22	340±29	344±28(↓3%)
Body weight gain, WG (g)					
Premating Day 1–70	73±18	70±19	70±23	65±23	59±25(↓20%)
Gestation Day 0–21	151±17	147±26	129±27	142±11	144±15(↓5%)
Lactation Day 1–22	26±24	20±20	21±21	23±19	29±15(↑11%)
F_1 Male					
Body weight (g)					
Body weight at end of premating (g)	517±49	547±44	519±45	510±38	463±35*(↓10%)
Day 121 body weight (g)	593±61	623±57	610±56	594±48	541±38*(↓ 9%)
Final body weight (g)	611±65	645±56	617±58	604±47	551±41*(↓10%)
Body weight gain, WG (g)					
Day 23-end of premating (g)	455±44	484±43	459±45	451±36	409±31*(↓10%)
Day 100-termination	69±18	69±20	66±15	65±14	55±14*(↓20%)
Day 23-termination	548±60	582±55*	557±58*	544±45	497±38*(↓9%)

Table 4. Summary of body weight and body weight gain data on P_1 and F1 parental male and female rats					
Parameter	**0 ppm**	**20 ppm**	**200 ppm**	**2000 ppm**	**20000 ppm**
F_1 Female					
Body weight (g)					
Body weight at end of premating (g)	292±24	300±24	301±27	285±22	274±20* (↓6%)
Body weight at start of gestation (g)	291±21	298±26	297±26	285±23	274±22*(↓6%)
Body weight at end of gestation (g)	451±35	475±41	469±18	450±33	440±37
Body weight at start of lactation (g)	335±31	344±32	342±35	333±27	317±26
Body weight at end of lactation (g)	353±21	361±28	355±28	349±29	340±27
Body weight gain, WG (g)					
Premating Day 23-Precohabitation	231±21	240±23	243±27	228±21	222±22
Gestation Day 0–21	160±28	173±22	168±14	159±17	167±27
Lactation Day 1–22	19±27	16±20	15±20	15±16	22±17

[a] Last value recorded before cohabitation. ( ): Value indicates relative to the control.
Data excerpted from the following pages of the study report: 86, 87 & 199-204 for P_1 generation; 405-408, 530-535 for F1 generation.

3. Food consumption and Food efficiency

The food consumption values were either slightly increased or comparable to those of the controls in all treatment groups of P_1 and F_1 parental animals (Tables 5), while slight food efficiency reduction was found in 20000 ppm P_1 males and females and F_1 males. No effect was seen in 20000 ppm females (Table 6). The food efficiency reduction in the 20000 ppm P_1 males and females and F_1 males might be treatment-related.

Table 5. Summary of food consumption for P_1 and F_1 parental male and female rats (g/kg/day)					
ppm	**0**	**20**	**200**	**2000**	**20000**
P_1 Males					
Day 1-92	55±3	55±2	55±3	55±2	56±2 (↑3%)
P_1 Females					
Premating Day 1–70	69±5	69±7	69±6	68±5	67±4 (↓3%)
Gestation Day 0–21	68±4	69±7	67±3	68±4	68±4
Lactation Day 1–15 [a]	139±15	136±16	135±15	141±10	139±11

Table 5. Summary of food consumption for P_1 and F_1 parental male and female rats (g/kg/day)					
ppm	**0**	**20**	**200**	**2000**	**20000**
F_1 Male					
Day 23-114	73±4	72± 2	73±2	75±4	79±4*(↑8%)
F_1 Female					
Premating Day 23-86[a]	99±5	97±5	101±6	101±7	106±7*(↑7%)
Gestation Day 0–21	72±7	71±6	74±6	74±7	76±5(↑6%)
Lactation Day 1–15 [a]	140±19	135±13	136±16	138±16	138±10

[a] Not determined for lactation days 15 to 22 as it is presumed the pups begin to consume feed after day 15
( ): Value indicates relative to the control.
Data excerpted from the following pages of the study: 89, 206, 209, & 212 for **P_1 generation and** 412, 537, 540, & 543 for **F_1 generation**.

Table 6. Summary of food efficiency for P_1 & F_1 parental male & female rats (g/day BWG/g/day FC*100)

ppm	0	20	200	2000	20000
P_1 Males					
Day 1-92	10±1	10±1	10±2	10±1	9±1 (↓9%)
P_1 Females					
Premating Day 1–70	6±1	5±1	5±1	5±2	5±2(↓16%)
Gestation Day 0–21	30±3	29±5	28±6	29±2	30±2
Lactation Day 1–15	5±2	4±3	4±3	6±2	4±2
F_1 Males					
Day 23-114	21±1	21±1	21±1	21±1	20±1*(↓5%)
F_1 Females					
Premating Day 23-86	18±1	19±1	18±1	18±1	18±1
Gestation Day 0–21	30±5	32±4	30±4	29±3	31±4
Lactation Day 1–15	4±4	5±3	5±2	5±2	5±2

* Statistically significant from control. ($p < 0.05$)
Data excerpted from the following pages of the study report: 90, 207, 210 & 213 for P_1 generation and 414, 538, 541, & 544 for F_1 generation.

4. Compound intakes

Compound intakes were presented for P_1 males and females and F_1 males and females parental animals at various intervals of the study (Tables 7). The premating compound intakes for P_1 females were used for the values for NOAEL and LOAEL, and they were presented in bold prints in Table 7.

Table 7. Compound intakes for P_1 & F_1 parental male & female rats (mg/kg bw/day)

ppm	0	20	200	2000	20000
P_1 Males					
Day 1-70[a]	0.0	1.2	11.4	115.0	1166.6
P_1 Females					
Premating Day 1–70	**0.0**	**1.4**	**13.9**	**136.0**	**1343.6**
Gestation Day 0–21	0.0	1.4	13.3	135.2	1352.7
Lactation Day 1–15[a]	0.0	2.7	27.0	282.6	2782.1
F_1 Males					
Day 23-114	0.0	1.4	14.6	150.8	1583.1
F_1 Females					
Premating Day 23-86[a]	0.0	1.9	20.1	202.8	2125.2
Gestation Day 0–21	0.0	1.4	14.7	148.5	1518.4
Lactation Day 1–15[b]	0.0	2.7	27.4	276.8	2769.0

[a] The last interval in which a substantial number of rats had a feed consumption value recorded before cohabitation.

[b] Not determined for lactation days 15 to 22 as it is presumed the pups begin to consume feed after day 15

* Statistically significant from control, $p < 0.05$

Data excerpted from the following pages of the report: 84, 192-194 for P_1 generation; 402, 523-525 for F1 generation.

5. Reproductive parameters, sperm evaluation and oestrus cycling

Cyantrniliprole produced no treatment-related effects on any of the mating and fertility parameters (numbers of days in cohabitation, mating index, fertility index, rats with confirmed mating dates during the first and second week of cohabitation and number of pregnancies per number of rats in cohabitation, implantation sites per delivered litter) at concentration levels as high as 20000 ppm on both P_1 and F_1 generations (Table 8).

Table 8. Reproductive indices for P_1 and F_1 parental data

ppm	0 ppm	20 ppm	200 ppm	2000 ppm	20000 ppm
P_1 Generation:					
No. pregnant/No. mated	25/30(83%)	25/30(83%)	28/30(93%)	26/30(87%)	21/29(70%)
Days in cohabitation	3.2	3.9	3.3	2.6	3.4
Mating index (%)	100	100	100	100	96.7
Fertility index (%)	83.3	83.3	93.3	86.7	72.4
Gestation length (days)	23.0	22.8	22.4	22.7	22.7
Number of implantation sites	373	342	375	374	304
Implantation site/dam	15.9±2.7	14.2±2.7	14.4±2.4	15.0±1.5	15.2±1.7
Dams delivered a litter [N(%)]	25 (100%)	24(100%)[a]	27(96%)	25(96%)[b]	21(100%)
Dams with stillborn pups	1	0	2	1	0
F_1 Generation:					
No. pregnant/No. mated	29/30(97%)	27/29(93%)	29/30(97%)	26/30(87%)	29/30(97%)
Days in cohabitation	3.9	3.9	3.2	2.6	3.7
Mating index (%)	100	100	100	100	100
Fertility index (%)	96.7	93.1	96.7	86.7	96.7
Gestation length (days)	22.7	22.6	22.5	22.6	22.6
Number of implantation sites	447	452	488	425	493
Dams with stillborn pups	3	1	6	0	4

Data excerpted from the following pages of the study report: 215-217 & 547-549.
[a] One dam was sacrificed on GD 22 due to adverse clinical observation.
[b] One dam was found dead on GD 23.
Mating index = no mated/no. cohabited.
Fertility index = no. pregnant/no. mated.

Sperm count, motility and morphology parameters were unaffected by the test substance at any concentrations in P_1 and F_1 males (Table 9). Values for number and percent motile sperm, number of non-motile sperm and total sperm count from the vas deferens and cauda epididymis and density were comparable among the test groups. Similarly, treatment-related effects were not seen in the abnormal sperm morphology which included measurements in number of detached heads, no head, broken flagellum, amorphous, coiled flagellum and bent flagellum.

The oestrous cycle evaluation of the P_1 and F_1 females showed no compound-related related effects at any treatment concentrations (Table 9).

Table 9. Summary data 0n Sperm parameter and estrous cycle					
ppm	**0**	**20**	**200**	**2000**	**20000**
Sperm parameter data					
P_1 generation:					
Vas deferens Sperm motility (%)	89.3	94.3	94.2	92.0	94.6
Sperm concentration (no./mL x 10^{-6})	1231±401	1259±625	1058±459	989±490	1031±447
Abnormal sperm morphology (%)	2.1	0.7	4.8	2.5	1.8
F_1 generation:					
Vas deferens Sperm motility (%)	93.9	90.1	91.7	90.9	88.1
Sperm concentration (no./mL x 10^{-6})	1143±688	1193±478	1317±508	1250±554	1259±597
Abnormal sperm morphology (%)	3.8	1.6*	1.8*	1.6*	2.5
Oestrous Evaluation					
P_1 generation:					
Mean estrus cycles/ 21 days	4.7±1.1	4.8±0.9	4.8±1.0	4.7±1.0	4.5±1.0
F_1 generation:					
Mean estrus cycles/ 21 days	4.5±1.3	4.8±1.2	4.4±1.1	4.3±1.2	4.7±1.2

*: Statistically significant decrease from control, $p<0.05$ and considered of no biological importance
Data excerpted from the following pages of the report: 99-100, 214, 425- 426, & 546.

3. Gross pathology, organ weights, and histopathology in parental animals

Cyantraniliprole did not produce compound-related gross lesions in P_1 and F_1 generation male and female rats.

Liver: Absolute and relative liver (relative to body weight or to brain weight) weights were increased in the 2000 ppm and 20000 ppm P_1 and F_1 generation males and females (Table 10). In most cases, the increases showed a statistically significant difference relative to the corresponding control group. The liver weight increases correlated with the increased incidence of hepatocelluar hypertrophy in the same dose groups (Tables 11 & 12). The changes in liver weight and associated increase in the incidence of hepatocellular hypertrophy were considered as adaptive effects.

Thyroid: In the 20000 ppm P_1 and F_1 generation males and in the 200, 2000, and 2000 ppm P_1 and F_1 generation females, there was a significant increase in the fixed thyroid lobes/parathyroid weights (absolute and relative) (Table 10). A corresponding dose-related increase in the incidence of the thyroid follicular epithelial cell hypertrophy/ hyperplasia was found in the 2000 and 20000 ppm P_1 males and females (Tables 11)

and in 200, 2000, and 20000 ppm F_1 males and females (Table 12). Note: ***There appears to be a difference in the terms used in describing the thyroid histopathology in the study report. The thyroid effect shown in the pathology report provided by Reseach Pathology Services, Inc. (starting from p. 974 of the study report) was hypertrophy/hyperplasia of the thyroid follicular epithelial cells. The individual animal data as presented in the pathology report are consistent with the narrative (page 984) of the pathology report. However, a second histopathology examination was conducted by Charles River Laboratories Preclinical Services, Pathology Associates, Frederick, MD. The summary tables as presented on pages 1637(P_1) & 1659 (F_1) of the study report show the thyroid finding to be "follicular cell hypertrophy" only. Interestingly, the study evaluation in the Tier II document submitted by the registrant (Tables 153 and 154 on pages 266 of the Tier II document) employed the term "thyroid follocular epithelium hypertrophy/hyperplasia". Under the circumstances, the US EPA primary reviewer is using the terms employed in the first pathology report, which describes the finding as "an increase in follicular epithelial cells (hyperplasia) and the thyroid epithelium was taller (hypertrophied) in the affected rats" (page 982 of the study report). Therefore, the increase in the incidence of thyroid follicular epithelial cell hyperplasia observed in this study was compound-related and adverse.***

Adrenal: In the adrenal cortex, an increased incidence of cell vacuolation was seen in 2000 and 20000 ppm P_1 males (Tables 11) and in 20, 200, 2000 and 20000 ppm F_1 males (Table 12). The adrenal cortical cell vacuolation in 20 and 200 ppm F_1 were all graded as minimal while only 2 incidence in 2000 or 20000 ppm were graded as mild. Although the adrenal cortical vacuolation was considered as treatment-related, adrenal cellular degeneration or toxicity was not present. The adrenal gland function was not affected by cyantraniliprole as shown in a study, where rats were treated with cyantraniliprole for 90-days and then challenged with ACTH, urine and serum corticosterone measurements were comparable to the controls (MRID 48119973). In addition, no increase in the incidence of adrenal cortical cell vacuolation was found in the long-term oral toxicity study using a more purified form of cyantraniliprole.. Therefore, the adrenal gland did not appear to be adversely affected either structurally or functionally. The absolute adrenal weight was slightly increased and showed a statistically significant difference from the control in the 2000 and 20000 ppm P_1 females and in the 200, 2000, and 20000 ppm F_1 females. However, the increase did not show a dose related-response (Table 10). In the absence of adverse changes in histopathology, the toxicological significance for the changes in adrenal weight in females was not clear.

Thymus: Absolute and relative thymus weights were decreased in the 2000 and 20000 ppm P_1 females and in the 20000 ppm F_1 females (↓35%) (Table 10). In the 2000 and 20000 ppm P_1 females, there was also an increase in the incidence of thymus atrophy (Table 11), but similar thymus effect was not seen in the P_1 males or F_1 females/males.

In the 20000 ppm F_1 females, there was a slight decrease in absolute but not relative brain and left ovary weights that were considered likely to be incidental and not

treatment related. Furthermore, while a slight decrease was seen in absolute and relative spleen weight in 20000 ppm F1 females, in the absence of histopathological changes in this organ the observed organ weight changes while treatment related are considered unlikely to be adverse.

Table 10. Parental Organ Weight Data

Ppm	0	20	200	2000	20000
Liver weight					
P_1 male					
Absolute weight (g)	23.5±3.0	23.9±2.9	23.4±4.1	25.2±2.9	26.1±3.4*
Relative to body weight (%)	3.6	3.6	3.6	4.0*	4.2*
Relative to brain weight (%)	982.2	1007.2	999.5	1065.0*	1102.1*
P_1 female					
Absolute weight (g)	15.8±2.0	15.5±1.6	15.6±1.4	18.8±2.4*	21.3±2.6*
Relative to body weight (%)	4.5	4.5	4.6	5.5*	6.2*
Relative to brain weight (%)	748.8	745.0	730.2	895.6*	1031.0*
F_1 male					
Absolute weight (g)	23.8±3.3	25.2±4.0	24.4±3.5	25. 5±3.1	25.3±2,9
Relative to body weight (%)	3.9	3.9	3.9	4.2*	4.6*
Relative to brain weight (%)	1041.1	1079.8	1053.0	1093.6	1132.0
F_1 females					
Absolute weight (g)	16.0±1.6	15.9±2.2	16.6±1.9	19.1±2.0**	21.2±2.6**
Relative to body weight (%)	4.5	4.4	4.7	5.5**	6.2**
Relative to brain weight (%)	752.2	739.8	780.4	897.6*	1043.0*
Thyroid/parathyroid weight					
P_1 males					
Absolute weight (g)	0.05±0.01	0.05±0.01	0.04±0.01	0.05±0.01	0.06±0.01* (↑20%)
Relative to body weight (% × 1000)	7.424	6.980	6.653*	7.588	8.892*
Relative to brain weight (%)	2.0	1.9	1.8	2.0	2.3*
P_1 females					
Absolute weight (g)	0.04±0.01	0.04±0.01	0.05.01*	0.05±0.01* (↑25%)	0.05±0.01* (↑25%)
Relative to body weight (% × 1000)	10.966	10.851	13.605*	15.006*	13.80*
Relative to brain weight (%)	1.8	1.8	2.2*	2.4*	2.3*

Table 10. Parental Organ Weight Data					
Ppm	**0**	**20**	**200**	**2000**	**20000**
F_1 males					
Absolute weight (g)	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01* (↑25%)
Relative to body weight (% × 1000)	7.088	7.139	7.509	7.563	8.827*
Relative to brain weight (%)	1.9	2.0	2.0	2.0	2.2*
F_1 females					
Absolute weight (g)	0.03±0.01	0.03±0.01	0.04±0.01* (↑33%)	0.04±0.01* (↑33%)	0.04±0.01* (↑33%)
Relative to body weight (% × 1000)	9.3	9.0	10.4*	10.5*	11.5*
Relative to brain weight (%)	1.5	1.5	1.7*	1.7	1.9*
Adrenal (left adrenal)					
F_1 Females					
Absolute weights (g)	0.04±0.01	0.04±0.01	0.05±0.01* (↑25%)	0.05±0.01* (↑25%)	0.05±0.01* (↑25%)
Relative to body weight (% x1000)	11.3	11.9	13.1*	14.4*	14.4*
Relative to brain weight (%)	1.9	2.0	2.2*	2.3*	2.4*
Thymus					
P_1 Females: absolute weights (g)	0.18±0.04	0.17±0.05	0.18±0.04	0.14±0.04* (↓22%)	0.13±0.04* (↓28%)
Relative to body weight (%)	0.05	0.05	0.05	0.04	0.04
Relative to brain weight (%)	8.8	8.3	8.5	6.6*	6.2*
F_1 Females: absolute weights (g)	0.26±0.06	0.27±0.07	0.28±0.08	0.22±0.06	0.17±0.06* (↓35%)
Relative to body weight (%)	0.07	0.08	0.08	0.06	0.06
Relative to brain weight (%)	12.1	12.7	13.1	10.6	8.6*

* Statistically significant from control ($p < 0.05$).
Data excerpted from pages 93-98, 223-225, 419-424, & 554-556 of the report.

Table 11. Incidences of microscopic effects, P_1 parental rats

ppm	0	20	200	2000	20000
Males:					
Liver Hypertrophy, hepatocellular, centrilobular	0/25	0/30	0/29	**28/30**	**29/29**
Thyroid Hypertrophy/hyperplasia, follicular epithelium	4/30	2/30	3/29	**19/30**	**28/30**
Adrenal gland Vacuolation, cortex	5/25	9/30	6/29	**11/30**	**18/29**
Females:					
Liver Hypertrophy, hepatocellular, centrilobular	1/29	0/29	0/30	**27/29**	**30/30**
Thyroid Hypertrophy/hyperplasia, follicular epithelium	3/29	5/28	5/30	**16/29**	**25/30**
Thymus Atrophy	1/29	2/29	2/30	**6/29**	**10/29**

Note: Bolded values were considered test substance-related changes.
Data excerpted from pages 1632-1638 of the study report.

Table 12. Incidences of microscopic effects, F_1 parental rats

ppm	0	20	200	2000	20000
Males:					
Liver Hypertrophy, hepatocellular, Centrilobular	0/30	0/30	0/29	**29/30**	**30/30**
Thyroid Hypertrophy/hyperplasia, follicular epithelium	1/30	2/30	**7/29**	**18/30**	**27/30**
Adrenal Gland Vacuolation, cortex	0/30	4/30	6/29	**10/30**	**11/30**
Females:					
Liver Hypertrophy, Hepatocellular, Centrilobular	1/30	0/29	0/29	**23/30**	**28/30**
Thyroid Hypertrophy/hyperplasia, follicular epithelium	1/30	1/29	**5/28**	**16/30**	**24/30**

Bolded values were considered test substance-related changes
Data excerpted from pages 1654-1661 of the study report

B. F_1 AND F_2 LITTER AND PUP DATA

1. General observations

A statistically significant increase in the incidence of mild dehydration among F_1 20000 ppm litters (97 observations/7rats vs 10 observation/2 rats for control) from birth to post-natal day (PND) 22. No clinical observations in the F_2 generation pups from birth to PND 22 were attributable to the test compound.

2. Litter size and pup survival

There were no test substance-related decreases in the number of pups born, born alive, alive on Days 5, 8, 15, or 22, and percent of male pups observed in the F_1 and F_2 litters (Table 13). There was a very slight, yet statistically significant increase in the number of F_1 pups found dead or presumed cannibalized on postnatal days 9 to 15 and 16 to 22 in the 20000 ppm exposure groups that resulted in a slight decrease in the percent survival from days 5-22 (lactation index) (96.4% as compared to 100% in controls). As 5/6 pups affected were from the same litter and the lactation value was within historical control range of the testing facility (range 85 to 100%), this minor reduction was not considered to be test substance-related.

For the F_2 generation, the mean numbers of liveborn pups in the 20000 ppm group were significantly increased relative to the controls. This increase is not considered to be test substance-related because a decrease rather than an increase would be a sign of toxicity. There also was a slight, but significant, increase in the number of stillborn F_2 pups observed at 20000 ppm (2.6%). This increase was within the historical control range (0-5%). The historical control data are presented in Attachment A.

Table 13. Litter size and pup survival for F_1 and F_2 rats

	Litter and pup data				
ppm	**0**	**20**	**200**	**2000**	**20000**
F_1 generation:					
Total No. of pups born	344	305	369	350	279
No. of litters (live pups)	25	24	27	25	21
Number pups born/litter	13.8	12.7	13.7	14.0	13.3
Number born alive/litter	13.7	12.7	13.5	14.0	13.3
Stillborn (%)	0.3	0.0	0.8	0.3	0.0
Day 1–5 survival (%) (Viability index)	97.7	98.7	97.5	99.1	97.5

Table 13. Litter size and pup survival for F_1 and F_2 rats					
	Litter and pup data				
ppm	**0**	**20**	**200**	**2000**	**20000**
Day 5–22 survival (%) (Lactation index)	100.0	100.0	100.0	100.0	96.4*
Male pups Day 1 (%)[a]	49.0	48.9	54.9	53.7	50.8
F2 generation:					
Total No. of pups born	425	423	473	403	465
No. of litters (live pups)	29	27	29	26	29
Number pups born/litter	14.6	15.7	16.3	15.5	16.0
Number born alive/litter	14.6	15.6	16.1	15.5	15.6*
Stillborn (%)	0.7	0.2	1.5	0.0	2.6*
Day 1–5 survival (%) (Viability index)	98.3	98.6	97.6	97.3	98.2
Day 5–22 survival (%) (lactation index)	100.0	99.5	99.6	100.0	99.6
Male pups Day 1 (%)[a]	47.5	49.5	49.9	53.0	48.0

* Significantly different from control, $p < 0.05$.
[a] Includes liveborn pups and pups that died before weighing on day 1 postpartum
Data excerpted from pages 216-219 & 548-549 of the report.

3. Pup body weight

There were no treatment-related effects on pup weights observed on the day of delivery in F_1 generation pups (Table 14). A statistically significant reduction in mean pup body weight per litter was observed on postnatal days 15 and 22 for F_1 litters in the 20000 ppm group, which corresponds to the time period when the pups typically begin to consume the rodent chow. Therefore, these body weight reductions were considered indicative of systemic toxicity resulting from the relatively large exposures that occurred during this time period.

Pup body weights were significantly reduced in the 20000 ppm exposure group of the F_2 litters at all intervals during the preweaning period (Table 14). Pup body weights per litter were also reduced in the 200 and 2000 ppm F_2 litters during most of the measuring interval. When litter size was used as a covariate in the analysis of covariance, pup weights in the 200 ppm group for the entire preweaning period and in the 2000 ppm group for days 1, 5, and 8 postpartum were not significantly different from control. Further, the mean pup body weights in the 200 and 2000 ppm groups at each interval during the preweaning period were within the laboratory's historical control range (day 1: 6.4-7.0g; day 5 preculling: 9.8-10.8 g; day 8: 16.4-18.2 g; day 15: 33.6-53.9 g; day

22: 52.6-59.7 g) (see Attachment A). Based on the historical control values, the observed F_2 pup body weight reductions at 200 would not be considered as adverse or treatment-related. Many pup body weight values at 2000 ppm were near the lower end of the historical control range, and therefore the pup body reduction seen at 2000 ppm was considered treatment related and adverse.

Table 14. Pup body weight/litter for F_1 & F_2 generations (gm)

ppm	0	20	200	2000	20000
F_1 generation					
At birth	7.0±0.6	7.1±1.2	6.8±0.6	6.7±0.7	7.0±0.8
Day 5 Postculling	10.7±1.6	12.0±2.7	10.8±1.1	10.3±1.3	10.9±1.3
Day 8	16.7±2.9	18.5±3.8	16.8±1.6	16.7±2.6	16.6±2.2
Day 15	34.7±5.4	35.7±5.0	32.8±4.6	33.7±4.7	31.0±6.2* (↓11%)
Day 22	56.7±7.9	57.5±6.4	53.4±6.5	53.6±6.6	49.0±7.8** (↓14%)
F2 generation					
At birth	6.8±07	6.5±0.6	6.3±0.7* [a] (↓7%)	6.3±0.7* [a] (↓7%)	6.1±0.5* (↓10%)
Day 5 Postculling	11.0±1.6	10.7±1.4	10.3±1.4 (↓6%)	9.9±1.4* [a] (10%)	9.6±1.0* (↓13%)
Day 8	18.2±2.0	17.8±2.3	16.7±1.7* [a] (↓8%)	16.7±2.0* [a] (↓8%)	15.5±1.6* (↓15%)
Day 15	37.5±2.9	37.1±3.4	35.0±2.4* [a] (↓7%)	34.7±3.0* (↓ 7%)	32.4±2.5 (↓14%)*
Day 22	59.7±4.9	57.6±5.2	55.9±4.3* [a] (↓6%)	55.4±4.5* (↓7%)	50.7±4.4* (↓15%)

* Significantly different from control, $p < 0.05$.

[a] Not statistically significant when litter size was used as a covariate in the analysis and within laboratory historical control range.

Data excerpted from pages 219 & 551 of the study report.

4. Gross pathology and organ weights

No test substance-related gross lesions were observed at necropsy in F_1 and F_2 litters.

Statistically significant decreases in thymus, spleen, adrenal, and brain weights were found in F_1 and F_2 pups (Table 14). Thymus weights were significantly reduced in 20000 ppm F_1 males and female (↓22%) relative to the controls; this reduction was also seen in both 2000 and 20000 ppm males and females of the F_2 generation. Similar

patterns were also seen in the spleen weight changes. The study report did not present the ratios of organ weight to body weight for F_2 pups; it contained only the ratios of selective organ weight to brain weight. The ratios for both thymus and spleen weight to brain weights showed statistically significant reduction in F_2 males and females of 2000 and 20000 ppm relative to the controls (Table 14a).

The brain weight was significantly decreased in 20000 ppm F_1 females (↓5%); brain weight reduction was also seen in 200, 2000, and 20000 ppm F_2 males and females, but the decrease in 200 and 2000 ppm was marginal (↓2-3%). Statistically significant decease in adrenal weight was seen in 20000 ppm females F_2 females. The registrant considered these organ weight reductions as a consequence of the body weight decrease in the pups. However, to establish the claim that organ weight decrease as a consequence of reduction in body weights, most of the organ weights should show reduction. For cyantraniliprole treatment pups, not all organs examined showed a reduction in weight.

Table 14. F_1 and F_2 Male and Female Pup Organ Weights (g/litter)

ppm	0	20	200	2000	20000
Thymus					
F_1 males	0.27±0.06	0.28±0.05	0.25±0.07	0.24±0.05	0.21±0.06* (↓22%)
F_1 females	0.27±0.05	0.28±0.04	0.24±0.04	0.24±0.06	0.21±0.06*(↓22%)
F_2 males	0.26±0.05	0.25±0.05	0.25±0.04	0.22±0.05*(↓15)	0.21±0.03*(↓19%)
F_2 females	0.27±0.05	0.26±0.05	0.26±0.04	0.23±0.05*(↓15%)	0.20±0.03*(↓26%)
Spleen					
F_1 males	0.29±0.07	0.30±0.05	0.27±0.06	0.25±0.06	0.26±0.18
F_1 females	0.30±0.07	0.30±0.07	0.27±0.05	0.26±0.06	0.22±0.07*(↓27%)
F_2 males	0.30±0.07	0.27±0.06*	0.28±0.06	0.26±0.05*(↓15%)	0.21±0.04*(↓30%)
F_2 females	0.30±0.06	0.26±0.06*	0.28±0.04	0.26±0.05*(↓15%)	0.22±0.04*(↓27%)
Brain					
F_1 males	1.60±0.10	1.63±0.09	1.59±0.11	1.61±0.11	1.55±0.14
F_1 females	1.54±0.11	1.60±0.09	1.54±0.09	1.55±0.08	1.47±0.14*(↓5%)
F_2 males	1.64±0.08	1.62±0.07	1.60±0.09*(↓2%)	1.59±0.07*(↓3%)	1.54±0.06*(↓5%)
F_2 females	1.60±0.07	1.58±0.07	1.56±0.06*(↓3%)	1.55±0.07*(↓3%)	1.51±0.07*(↓6%)
Adrenal					
F_2 males	0.019±0.005	0.019±0.004	0.018±0.003	0.019±0.004	0.017±0.003
F_2 females	0.020±0.005	0.018±0.003	0.018±0.002	0.017±0.004	0.016±0.004*(↓20%)

* Statistically significant ($p<0.05$) ** Statistically significant ($p<0.05$)

Data excerpted from pages 227-230 & 559-562 of the report.

Table 14a. Relative Thymus and Spleen to Brain weights For F2 generation males and females[a]					
ppm	**0**	**20**	**200**	**2000**	**20000**
Thymus					
F_2 males (%)	15.76	15.32	15.9	14.01*	13.37**
F_2 females (%)	16.77	16.43	16.84	14.95*	13.25**
Spleen					
F_2 males (%)	18.30	16.48	17.46	16.25*	13.63*
F_2 females (%)	19.13	16.70	18,09	16.58**	14.39**

* Statistically significant ($p<0.05$) ** Statistically significant ($p<0.05$)

[a]**: For the F2 pups, Only organ weight to brain weight data were presented in the study report.**

Data excerpted from page 561 of the study report.

5. F_1 sexual maturation

Despite there being a statistically significant reduction in body weight at the time of preputial separation, dietary exposure to the test substance did not affect the average days of achievement in the F_1 generation male rats. In F_1 female rats, the average date on which vaginal patency occurred was delayed (2.2 days) in the 20000 ppm exposure group relative to the control group (Table 15). However, the mean value of 35.1 days in this test group was within the testing laboratory's historical control range (mean 32.7 days; range 30.1 to 35.3 days) (see Attachment A). Further, the delay was not statistically significant when individual body weight was used as a covariate in the analysis of covariance, indicating that the delay in time to vaginal patency was a generalized effect on overall body weight that was evident in the F_1 generation female rats at weaning and persisted postweaning.

Table 15. Sexual maturation parameters F_1 generation male and female rats

ppm	0	20	200	2000	20000
F1 generation					
Preputial separation (Day)	48.3±2.0	47.8±2.4	47.9±2.3	47.9±1.8	49.3±3.1
Vaginal opening (Day)	32.9±2.2	33.9±2.4	33.6±2.1	33.7±2.8	35.1±2.5[a**]

a Mean value within historical control range and not significantly different from control, when individual body weight was used as a covariate in analysis.

** Statistically significant from the control ($p\leq0.01$).

Data excerpted from pages 415 (males) & 545 (females) of the study report.

III. CONCLUSION

Under the conditions of the study, cyantraniliprole produced treatment-related effects at dietary concentrations of 200, 2000 and 20000 ppm. For the parental animals, reductions in body weights and body weight gains at 20000 ppm (P_1 and F_1 generation male and female rats) were seen. A significant increase in the fixed thyroid lobes/parathyroid weights (absolute and relative) was seen in the 20000 ppm P_1 and F_1 males and in 200, 2000, and 2000 ppm P_1 and F_1 females. A correlated and dose-related increase in the incidence of the thyroid thyroid follicular cell hypertrophy/hyperplasia was also found in the 2000 and 20000 ppm P_1 males and females and in 200, 2000, and 20000 ppm F_1 males and females. In addition, significant reductions in thymus weight accompanied by thymus atrophy were noted in 2000 and 20000 ppm P_1 generation female rats (thymus weight: ↓22% - ↓28%). Thymus weight in 20000 ppm F_1 females was also decreased (↓35%).

No treatment-related reproductive effects were found. The data showed that estrous cyclicity, sperm analyses, mating behavior, conception and fertility, parturition, gestation length, lactation, weaning and the growth and development of offspring including onset of puberty were all comparable across the test groups.

For the offspring, treatment-related effects included decreased body weights at 20000 ppm in the F_1 generation pups on PNDs 15 and 22, and in F_2 generation pups at 2000 and 20000 pm from birth to PND 22. Pup thymus and spleen weights were deceased in 20000 ppm F1 males and in 2000 and 20000 ppm F2 males and females. Decreases in 20000 ppm F_1 and F_2 pup brain weight and F2 pup adrenal weight were also found. An increase in the number of 20000 ppm F_1 pups with dehydration was also seen.

The **parental NOAEL** was 20 ppm (1.4 mg/kg/day). LOAEL was 200 ppm (14 mg/kg/day) based on organ weight and histopathology changes in the thyroid gland (thyroid follicular epithelial cell hypertrophy/hyperplasia) in F_1 generation adult male and/or female rats. It should be noted that data on of ≥ 2000 ppm test animals were more robust in terms of number and severity of effect in demonstrating the toxicity of cyantraniliprole.

The **reproductive and fertility NOAEL** was 20000 ppm (1343.6 mg/kg/day) (the highest dietary concentration tested) based on the lack of adverse test substance-related effects on fertility and reproductive parameters at any dose levels in the study. The **reproductive and fertility LOAEL** was greater than 20000 ppm

The **offspring** NOAEL was 200 ppm (13.3 mg/kg/day). LOAEL was 2000 ppm (20.1 mg/kg/day) based on dose related decreases in organ weights (thymus and spleen).and decreased pup body weight of the F_2 generation.

This study is classified as fully reliable (acceptable/guideline) and satisfied the guideline requirements (OPPTS 870.3800; OECD N0. 416; MAFF 12 Nousan 8147) for a reproduction and fertility study.

Attachment A. Historical Control Data

SUMMARY OF NATURAL DELIVERY OBSERVATIONS
Crl:CD(SD) RATS
(STUDIES WITHOUT CULLING)

PERIOD	JUNE 2007 - JUNE 2009			
RATS ASSIGNED TO NATURAL DELIVERY	N	555		
PREGNANT	N (%)	506 (89.2)		
DELIVERED LITTERS	N (%)	505 (98.5)		
			RANGE	# STUDIES INCLUDED
DURATION OF GESTATION IN DAYS	MEAN	22.7	(22.2-24.0)	33
IMPLANTATION SITES PER DELIVERED LITTER	MEAN	14.9	(7.8-16.7)	33
DAMS WITH STILLBORN PUPS	%	7.3	(0-33.3)	33
DAMS WITH NO LIVEBORN PUPS	%	0.1	(0-4.2)	33
GESTATION INDEX (# RATS WITH LIVEBORN/# PREGNANT RATS)	%	97.2	(50.0-100.0)	33
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	%	0.2	(0-3.7)	18
DAMS WITH ALL PUPS DYING DAYS 1-5 POSTPARTUM	%	0.0	---	15
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	%	0.6	(0-10.0)	17
PUPS DELIVERED (TOTAL)	MEAN	14.0	(7.5-15.8)	33
LIVEBORN	MEAN	13.9	(7.5-15.7)	33
	%	99.3	(95.0-100)	
STILLBORN	MEAN	0.1	(0-0.7)	33
	%	0.7	(0-5.0)	
UNKNOWN VITAL STATUS	N	0.0	(0-1)	29

Data taken from page 2296 of the study report.

SUMMARY OF NATURAL DELIVERY OBSERVATIONS
Crl:CD(SD) RATS
(STUDIES WITHOUT CULLING)

		MEAN OR %	RANGE	# STUDIES INCLUDED
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED (CONT.)				
Day 5	MEAN	46.7	(37.8-56.0)	17
Day 7	MEAN	49.7	(42.8-55.2)	17
Day 14	MEAN	49.5	(46.7-53.4)	15
Day 21	MEAN	49.1	(40.9-53.4)	15
Day 28	MEAN	48.1	---	1
LIVE LITTER SIZE AT WEIGHING				
Day 1	MEAN	13.8	(7.2-15.7)	33
Day 4	MEAN	13.9	(12.2-15.4)	25
Day 5	MEAN	13.5	(7.2-16.2)	16
Day 7	MEAN	13.6	(12.0-14.9)	17
Day 14	MEAN	13.6	(13.0-14.7)	15
Day 21	MEAN	13.7	(13.0-14.6)	15
Day 28	MEAN	14.6	---	1
PUP WEIGHT/LITTER (GRAMS)				
Day 1	MEAN	6.7	(6.1-7.5)	33
Day 4	MEAN	9.3	(8.0-10.2)	25
Day 5	MEAN	10.6	(9.8-11.9)	15
Day 7	MEAN	13.4	(11.9-14.9)	17
Day 14	MEAN	26.1	(23.1-28.2)	15
Day 21	MEAN	40.7	(37.1-43.5)	15
Day 28	MEAN	75.4	---	1

Data taken from page 2298 of the study report.

SUMMARY OF NATURAL DELIVERY OBSERVATIONS
Crl:CD(SD) RATS
(STUDIES WITH CULLING)

PERIOD	JUNE 2007 - JUNE 2009			
RATS ASSIGNED TO NATURAL DELIVERY	N	239		
PREGNANT	N (%)	221 (92.7)		
DELIVERED LITTERS	N (%)	217 (99.5)		
			RANGE	# STUDIES INCLUDED
DURATION OF GESTATION IN DAYS	MEAN	22.7	(22.5-23.0)	9
IMPLANTATION SITES PER DELIVERED LITTER	MEAN	15.1	(14.2-16.2)	9
DAMS WITH STILLBORN PUPS	%	9.2	(3.7-17.6)	9
DAMS WITH NO LIVEBORN PUPS	%	0.0	---	9
GESTATION INDEX (# RATS WITH LIVEBORN/# PREGNANT RATS)	%	98.1	(87.0-100)	9
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	%	0.0	---	9
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	%	0.0	---	5
DAMS WITH ALL PUPS DYING DAYS 6-22 POSTPARTUM	%	0.0	---	2
PUPS DELIVERED (TOTAL)	MEAN	14.2	(13.2-15.3)	9
LIVEBORN	MEAN	14.0	(13.0-15.2)	9
	%	99.0	(96.5-99.8)	
STILLBORN	MEAN	0.1	(0-0.5)	9
	%	1.1	(0.2-3.5)	
UNKNOWN VITAL STATUS	N	0.0	---	9

Data taken from page 2299 of the study report.

SUMMARY OF NATURAL DELIVERY OBSERVATIONS
Crl:CD(SD) RATS
(STUDIES WITH CULLING)

		MEAN OR %	RANGE	# STUDIES INCLUDED
LIVE LITTER SIZE AT WEIGHING (CONT.)				
Day 5 (Preculling)	MEAN	14.0	(12.8-15.2)	5
Day 5 (Postculling)	MEAN	7.9	(7.8-8.0)	5
Day 7 (Preculling)	MEAN	9.2	(7.8-13.0)	4
Day 7 (Postculling)	MEAN	8.0	---	1
Day 8	MEAN	7.8	(7.8-8.0)	5
Day 14	MEAN	7.9	(7.8-8.0)	4
Day 15	MEAN	7.8	(7.8-7.9)	5
Day 21	MEAN	7.9	(7.8-8.0)	4
Day 22	MEAN	7.8	(7.8-7.9)	5
PUP WEIGHT/LITTER (GRAMS)				
Day 1	MEAN	6.7	(6.4-7.0)	9
Day 4 (Preculling)	MEAN	9.0	(8.7-9.3)	4
Day 4 (Postculling)	MEAN	9.1	(8.9-9.4)	3
Day 5 (Preculling)	MEAN	10.4	(9.8-10.8)	5
Day 5 (Postculling)	MEAN	10.6	(10.2-11.0)	5
Day 7 (Preculling)	MEAN	13.9	(12.3-15.2)	4
Day 7 (Postculling)	MEAN	12.4	---	1
Day 8	MEAN	17.2	(16.4-18.2)	5
Day 14	MEAN	31.0	(29.6-33.1)	4
Day 15	MEAN	34.8	(33.6-37.5)	5
Day 21	MEAN	49.9	(46.8-53.9)	4
Day 22	MEAN	55.3	(52.6-59.7)	5

Data taken from page 2302 of the study report.

SEXUAL MATURATION HISTORICAL CONTROL DATA

SUMMARY

	AVERAGE	MINIMUM	MAXIMUM	# STUDIES INCLUDED
MALE RATS	27.6	10	72	150
Preputial Separation (Avg. day postpartum obs.)	46.2	41.333	49.7	150
Body Weight (G) on day of sexual maturity	235.7	207.5	277.8	52
Body Weight (G) measured weekly	216.1	208.7	223.5	2
FEMALE RATS	27.5	10	80	152
Vagina Patent (Avg. day postpartum obs.)	32.7	30.1	35.3	152
Body Weight (G) on day of sexual maturity	105.2	92.4	120.8	53
Body Weight (G) measured weekly	105.4	90.7	120.1	2

Data taken from page 2303 of the study report.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.6.10 Developmental oral toxicity study in the rat

Report: Munley, S.M. (2009); DPX-HGW86 technical: Developmental toxicity in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Testing Facility Report No. DuPont-19188. April 17, 2009. MRID 48119968. Unpublished.

Guidelines: OPPTS 870.3700 (1998)
OECD 414 (2001),
Directive 87/302/EEC Part B,
MAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed statements of GLP, Quality Assurance, and Data Confidentiality were presented in the report.

Executive summary:

In a developmental toxicity study (MRID 48119968), groups of time-mated Crl:CD®(SD) female rats (22/dose group) received cyantraniliprole (94.5%; HGW86-230) by oral gavage at doses of 0, 20, 100, 300, or 1000 mg/kg bw/day on gestation Days 6 to 20. Cyantraniliprole was suspended in 0.5% aqueous methylcellulose, and the dose volume was 5 mL/kg bw. For the dams, the following parameters were evaluated: body weight, body weight gain, food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Fetal parameters evaluated were the following: fetal body weight, incidences of dead fetuses and/or fetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

No test substance-related effects on maternal clinical observations, body weight, weight gain, food consumption or gross post-mortem observations were observed at any dose level. Unscheduled mortality did not occur. The mean number of corpora lutea, implantation sites, resorptions, live fetuses, fetal weight and sex ratio were comparable across all groups. There were no test substance-related fetal external, visceral or skeletal malformations or variations.

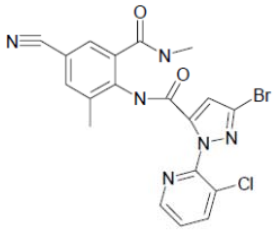
The maternal and developmental NOAELs were 1000 mg/kg bw/day, the highest dosage tested. LOAELs were not established. With oral administration, 1000 mg/kg/day is the limit dose for a developmental toxicity study.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for a developmental toxicity study in rats **(**OPPTS 870.3700; OECD 414; Directive 87/302/EEC Part B; MAFF 12 Nousan 8147).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Off-white solid
CAS #:	736994-63-1
Stability of test compound:	The test substance was mixed homogeneously, was present at the targeted concentrations and was stable for up to 8 days at room temperature.

2. Vehicle and/or positive control: 0.5% Aqueous methylcellulose
3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)
Age at dosing:	Approximately 67 days old
Weight at dosing:	Approximately 222–266 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	2–5 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   June 01, 2008 to December 05, 2008

2. Animal assignment and treatment

   In a previous pilot study in rats, cyantraniliprole was administered by oral gavage to time-mated Crl:CD®(SD) female rats (10/dose group) on gestation Days 6 to 20 at dosages of 100, 500, or 1000 mg/kg bw/day (the pilot study was submitted). Test substance-related findings were limited to reductions in body weight and food consumption and increases in alopecia at 1000 mg/kg bw/day. Based on these results, doses of 0, 20, 100, 300, and 1000 mg/kg bw/day were selected for the main study (Table 1). The test substance was administered in 0.5% aqueous methylcellulose at a volume of 5 mL/kg bw based on the most recent body weight. A negative control group received 0.5% aqueous methylcellulose alone. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1 Study design

Group no.	Exposure (mg/kg bw/day)[a]	Test formulation concentration (mg/mL)[b]	Number of time-mated females
1	0[c]	0	22
2	20	4	22
3	100	20	22
4	300	60	22
5	1000	200	22

[a] Formulations of test substance in 0.5% aqueous methylcellulose were administered once daily by oral gavage on Days 6–20G at a dosing volume of 5 mL/kg bw.

[b] To achieve these concentrations of active ingredient, the formulations were adjusted for sample purity (94.5%)

[c] The control group received 0.5% aqueous methylcellulose only at 5 mL/kg bw.

3. Dosing suspensions, preparation, and analysis

   Suspensions of test substance in 0.5% aqueous methylcellulose were prepared fresh on each day of dosing until stability was established for 8 days at room temperature. The stability, homogeneity, and concentration of cyantraniliprole in the dosing suspensions were checked by analyses using HPLC near the beginning and end of the study. In dose samples prepared on 01 June 2008, the test substance was at target concentrations ±3.5% and homogeneous (CVs = 2%, 0%, 4%, and 1%, respectively, for 4-, 20-, 60-, and 200-mg/mL samples). In dose samples prepared on 13 June 2008, the test substance was at target concentrations ±4.5%. In dose samples prepared on 01 June 2008 and stored at room temperature for 8 days then analyzed, the test substance was at target concentrations ±10.3%.

4. Statistics: The statistical methods employed are presented in Table 2. For litter parameters, the proportion of affected fetuses per litter or the litter mean was used as the experimental unit for statistical evaluation. The level of significance selected was $p < 0.05$.

Table 2. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Maternal body weight, maternal body weight gain, maternal food consumption, *corpora lutea*, live foetuses, dead foetuses, resorptions, implantations	Levene's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance and Dunnett's test	Kruskall-Wallis test followed by Dunn's test
Incidence of pregnancy, maternal mortality, females with total resorptions, early deliveries	None	Cochran-Armitage test[b]	
Incidence of foetal alterations	None	Exact Mann-Whitney with a Bonferroni-Holm adjustment	
Foetal weight (covariates: litter size, sex ratio), sex ratio (covariate: litter size)	Levene's test for homogeneity and Shapiro-Wilk test for normality[c]	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance

[a] If the Shapiro-Wilk test was not significant, but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskall-Wallis test is followed with Dunn's test.

[b] If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.

[c] A normalizing, variance stabilizing transformation was used as needed.

C. METHOD

1. Observations

   Clinical signs were recorded once on Day 4G and twice daily on Days 6 to 20G.

2. Body weights

   All dams were weighed once on Day 4G and daily on Days 6 to 21G.

3. Food consumption

   Food consumption was measured on Days 4, 6, 8, 10, 12, 14, 16, 18, 20, and 21G.

4. Sacrifice and pathology

   At termination (gestation Day 21), animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Viscera were examined. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, and corpora lutea counts, and pup sex ratio.

6. Evaluation of fetuses

Parameters evaluated in fetuses were body weight, incidences of dead fetuses and/or fetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

No treatment-related clinical signs of toxicity were observed in any test group.

2. Mortality

There was no test substance-related effect on survival.

3. Body weight, body weight gain, and food consumption

There were no adverse test substance-related effects on body weights or body weight gains (Table 3).

Table 3 *. Summary of maternal body weight, body weight gain, and food consumption

	Dose (mg/kg bw/d)				
Parameter	**0** **n-22**	**20** **n-22**	**100** **n=21**	**300** **n=21**	**1000** **n=21**
Body weight day 21 (g)	408.8±26.0	399.9±23.4	409.1±26.0	396.3±31.7	400.7±20.5
Adjusted[a] body weight day 21 (g)	311.0±18.0	304.5±15.5	311.9±15.6	302.5±16.6	300.5±15.6
Body weight gain 6–21G (g)	165.1±20.8	157.8±19.4	162.0±22.5	157.1±27.5	160.8±15.1
Adjusted[a] body weight gain 6–21G (g)	67.3±13.7	62.4±12.3	64.9±12.0	63.3±12.6	60.9±11.5
Food consumption 6–21G (g/day)	29.9±2.4	28.9±2.1	29.6±2.0	28.7±2.7	28.8±2.1

[a] Weight change using final body weight minus products of conception

Note: There were no statistically significant differences at $p<0.05$

* Data in this table obtained from pages 27 – 31 of the study report.

4. Reproductive outcomes

There were no adverse test substance-related effects on reproductive parameters or litter data endpoint during the course of this study (Table 4).

Table 4 *. Reproductive outcome

Parameter	Dose (mg/kg bw/d)				
	0	**20**	**100**	**300**	**1000**
Number pregnant	22	22	21	21	21
Number delivered early	0	0	0	0	0
Number with total resorptions	0	0	0	0	0
Mean corpora lutea	13.4±2.4	13.6±1.6	13.3±1.6	13.5±2.2	14.4±2.5
Mean implants/Litter	12.7±2.4	12.7±1.3	12.9±1.2	12.7±1.8	13.7±2.0
Mean resorptions/Litter	0.32±0.65	0.14±0.35	0.48±1.03	0.57±1.36	0.48±1.03
Mean live fetuses/Litter	12.4±2.2	12.6±1.4	12.4±1.5	12.1±2.8	13.2±1.1
Mean dead fetuses/Litter	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Fetal sex ratio[a]	0.49±1.4	0.48±0.20	0.48±0.15	0.54±0.14	0.50±0.16
Mean fetal weight (g)	5.86±0.20	5.65±0.38	5.83±0.34	5.67±0.22	5.69±0.26

[a] Number of male foetuses/total foetuses per litter

Note: There were no statistically significant differences at $p < 0.05$.

* Data in this table obtained from pages 33 and 34 of the study report.

5. Gross pathology

No test substance-related gross lesions were observed at necropsy.

B. FETAL EFFECTS

No test substance-related effect on fetal viability, fetal weight or the incidence of fetal external, visceral, or skeletal malformations or variations was detected (Table 5).

Table 5. Summary of Fetal Effects

Parameter	Dose (mg/kg bw/d)				
	0	**20**	**100**	**300**	**1000**
No. Examined (external/skeletal)	273 (22)	277 (22)	260 (21)	255 (21)	278 (21)
No. Examined (visceral/head)	132 (22)	134 (22)	126 (21)	122 (21)	135 (21)
Malformations					
Gastroschisis	1 (1)				
Micromelia, limb	1 (1)				
Brachydactyly, digit	1 (1)				
Protruding tongue	1 (1)	1 (1)			
Cleft palate	1 (1)				
Enlarged kidney					1 (1)
Short tibia	1 (1)				

Parameter	Dose (mg/kg bw/d)				
	0	20	100	300	1000
Short fibula	1 (1)				
Variations					
Dilated ureter					1 (1)
Misshapen liver		1 (1)			
Incomplete ossification, frontal skull				3 (1)	
Incomplete ossification, zygomatic skull	1 (1)	1 (1)	2 (2)	3 (1)	
Incomplete ossification, interparietal skull	1 (1)	1 (1)	1 (1)	5 (2)	1 (1)
Incomplete ossification, supraoccipital skull	1 (1)			7 (3)	
Incomplete ossification, parietal skull	2 (2)	1 (1)	4 (3)	5 (2)	
Incomplete ossification, pubis	1 (1)			1 (1)	
Incomplete ossification, ischium				1 (1)	
Incomplete ossification, cervical arch				1 (1)	
Misaligned lumbar centrum				1 (1)	1 (1)
Bipartite ossification, thoracic centrum	2 (2)	9 (6)	5 (4)	8 (4)	2 (2)
Short rib	1 (1)	1 (1)			
Cervical rib	4 (3)		4 (3)		1 (1)
Full supernumerary rib			1 (1)		
Short supernumerary rib	3 (2)	2 (2)	1 (1)	5 (5)	4 (2)
Extra ossification site, rib	13 (6)	5 (3)	10 (4)	20 (8)	10(8)
Unossified sternebrae	2 (2)				
Misaligned sternebrae	1(1)				1 (1)
Fused sternebrae	1 (1)				
Bipartite ossification sternebrae	1 (1)	1 (1)			
Incomplete ossification sternebrae					1 (1)

Note: Numbers examined and affected are presented as foetuses (litters). Statistical analyses are only conducted on the individual endpoints; the overall totals are presented for information only.
There were no statistically significant differences (mean percent affected per litter) from control at $p < 0.05$.
Data in this table obtained from pages 35 – 41 of the study report.

III. CONCLUSION

Under the conditions of this study, no compound-related effects were found in the dams and fetuses at dose levels as high as 1000 mg/kg bw/day. With oral administration, 1000 mg/kg/day is the limit dose for a developmental toxicity study. The NOAELs for both maternal and developmental toxicity were 1000 mg/kg bw/day (highest dose tested). LOAELs were not established.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.6.11 Developmental oral toxicity study in the rabbit

IIA 5.6.11/01

Report: Munley, S.M. (2009); DPX-HGW86 technical: Developmental toxicity study in rabbits. DuPont Haskell Laboratories, Newark, Delaware, USA. Testing Facility Report No. DuPont-19189. July 12, 2009. MRID 48119969. Unpublished.

Guidelines: OPPTS 870.3700 (1998)
OECD (Part 414) (2001)
Directive 87/302/ECC 87/302/EEC, Part B.
MAFF 12 Nousan 8147 (2000)
Deviations: None

GLP: Yes Signed statements of GLP, Quality Assurance, and Data Confidentiality were presented in the report.

Executive summary:

In a developmental toxicity study MRID 48119969), cyantraniliprole technical (94.5%; HGW86-230) was orally administered by gavage to time-mated Hra:(NZW)SPF female rabbits (22/dose group) on gestation Days 7 to 28. Doses were prepared as a suspension in 0.5% aqueous methylcellulose, and they were 0, 25, 100, 250, or 500 mg/kg bw/day. The dose volume was 5 mL/kg bw. For the dams the following parameters were evaluated: body weight, body weight gain (absolute and adjusted for the gravid uterus), food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. The following fetal parameters were evaluated: body weight, incidences of dead fetuses and/or fetal resorptions, and incidences of external, visceral, and skeletal malformations and variations.

At 100 mg/kg bw/day and above, increased clinical signs of toxicity such as diarrhea, and decreased body weights and food consumption were found. Maternal toxicity was sufficiently severe to result in the early sacrifice of two does at 100 mg/kg bw/day. Prior to the schedule sacrifice, 4 does at 250 mg/kg bw/day and 3 does at 500 mg/kg bw/day groups had to be sacrificed in extremis due to marked decreases in body weight and in food consumption. At 250 and 500 mg/kg bw/day, abortions in late gestation and/or early deliveries on the day of scheduled termination occurred. The affected does had prolonged periods of markedly reduced food consumption and subsequent body weight losses. Therefore, abortions/early deliveries were influenced by maternal toxicity that was observed at these dose levels. **The maternal no-observed-adverse-effect level (NOAEL) was 25 mg/kg bw/day. The maternal LOAEL was 100 mg/kg bw/day based on increased mortality, increased incidences of diarrhea and reduced and/or absent of feces, decreased body weights, and reduced food consumption.**

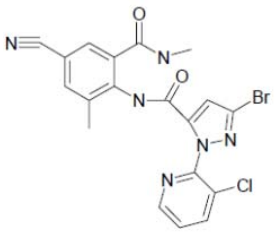
The developmental effect was demonstrated by test substance-related reductions in mean fetal body weights at 250 and 500 mg/kg bw/day. No treatment-related fetal malformations or variations were found. **The developmental NOAEL was 100 mg/kg bw/day. The developmental LOAEL was 250 mg/kg bw/day based on reductions in mean fetal body weights.**

This study is fully reliable (acceptable/guideline) and satisfies the requirements for a developmental toxicity study in rabbits **(**OPPTS 870.3700; OECD 414; Directive 87/302/EEC Part B, MAFF 12 Nousan 8147).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Off-white solid
CAS #:	736994-63-1
Stability of test compound:	The test substance was mixed homogeneously, was present at the targeted concentrations and was stable for up to 8 days at room temperature.

2. Vehicle and/or positive control: 0.5% aqueous methylcellulose

3. Test animals

Species:	Rabbit
Strain:	Hra:(NZW)SPF female rabbits
Age at dosing:	Approximately 5.5–6 months old
Weight at dosing:	2.8290–3.6777 kg
Source:	Covance Research Products, Denver, Pennsylvania
Acclimation period:	Not specified
Diet:	PMI® Nutrition International, LLC Certified Rabbit LabDiet® (#5322), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	16–22°C

Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   June 22, 2008 to September 22, 2008

2. Animal assignment and treatment

   In a developmental toxicity study, cyantraniliprole was administered by oral gavage to mated Hra:(NZW)SPF female rabbits (22/dose group) on gestation Days 7 to 28. Based on a pilot developmental toxicity study in rabbits, doses of 0, 25, 100, 250, or 500 mg/kg bw/day were selected for this study (Table 1). It should be noted that the pilot study was not submitted. The test substance was administered in 0.5% aqueous methylcellulose at a volume of 5 mL/kg bw based on the most recent body weight. A negative control group received 0.5% aqueous methylcellulose alone. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study design

Group no.	Exposure (mg/kg bw/day)[a]	Test formulation concentration (mg/mL)[b]	Number of time-mated females
1	0[c]	0 (control)	22
2	25	5	22
3	100	20	22
4	250	50	22
5	500	100	22

[a] Formulations of test substance in 0.5% aqueous methylcellulose were administered once daily by gavage on GD 7-28 at a dosing volume of 5 mL/kg.

[b] To achieve these concentrations of active ingredient, the formulations were adjusted for sample purity (94.5%)

[c] The control group animals received vehicle (0.5% aqueous methylcellulose) only at a dosing volume of 5 mL/kg bw.

3. Dosing suspensions, preparation and analysis

   Dosing formulations were prepared and used within the range of established stability. The stability, homogeneity, and concentration of cyantraniliprole in the dosing suspensions were checked by analyses using HPLC near the beginning and end of the study. In dose samples prepared on 20 June 2008, the test substance was at target concentrations ±5.0% and homogeneous (RSDs = 1%, 2%, 3%, and 2%, respectively, for 5-, 20-, 50-, and 100-mg/mL samples). In dose samples prepared on 07 July 2008, the test substance was at target concentrations ±7.0%. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

4. Statistics: Statistical methods employed in this study are presented in Table 2.

Table 2. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Maternal body weight, maternal body weight gain, maternal food consumption, *corpora lutea,* live foetuses, dead foetuses, resorptions, implantations	Levene's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance followed with Dunnett's test	Kruskall-Wallis test followed with Dunn's test
Incidence of pregnancy, maternal mortality, females with total resorptions, early deliveries	None	Sequential application of Cochran-Armitage test[b]	
Incidence of foetal alterations	None	Exact Mann-Whitney with a Bonferroni-Holm adjustment	
Foetal weight (covariates: litter size, sex ratio), sex ratio (covariate: litter size)	Levene's test for homogeneity and Shapiro-Wilk test for normality[c]	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance

[a] If the Shapiro-Wilk test was not significant, but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskall-Wallis test is followed with Dunn's test.

[b] If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.

[c] A normalizing, variance stabilizing transformation was used as needed.

For litter parameters, the proportion of affected foetuses per litter or the litter mean was used as the experimental unit for statistical evaluation. The level of significance selected was $p < 0.05$.

C. METHODS

1. Observations

   Clinical signs were recorded once during the quarantine and pretest period, and twice daily on Days 7 to 28G.

2. Body weights

   All dams were weighed daily.

3. Food consumption

   Food consumption was measured daily.

4. Sacrifice and pathology

   At termination (GD 29), animals were sacrificed by intravenous injection of an approved commercial euthanasia solution (Beuthanasia-D) and exsanguination. Viscera were examined. The uterus of each pregnant rabbit was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes and evaluation of foetuses

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, *corpora lutea* counts, and pup sex ratio. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

Cyantraniliprole produced the following treatment-related clinical signs: diarrhea at 100 mg/kg bw/day and above, reduced or absent feces at 100 mg/kg bw/day and above, and stained fur at 500 mg/kg bw/day (Table 3). The duration of observations for both diarrhea and reduced or absent of feces showed a dose-related response. There were no test substance-related clinical observations at 25 mg/kg bw/day (Table 3).

Table 3. Clinical signs of toxicity

Observations	Dose (mg/kg/day)				
	0	**25**	**100**	**250**	**500**
Diarrhea					
Incidence	3/22	2/22	6/22	12/22	8/22
No. of observations	4	2	26	31	24
Duration of observation (From days –to-)	10-20	27-27	10-29	16-29	13-29
Reduced or absent of feces					
Incidence	0/22	0/22	1/22	4/22	6/22
No. of observations	0	0	1	9	12
Duration of observation (From days –to-)	--	--	26-26	19-28	17-28
Stained skin/fur—brown-tail perineum					
Incidence	3/22	1/22	4/22	4/22	9/22
No. of observations	38	4	22	21	54
Duration of observation (From days –to-)	12-29	25-28	21-29	17-29	16-29

Data excerpted from pages 27-29 of the study report.

2. Mortality

Maternal toxicity was sufficiently severe to result in the early sacrifice of two does at

100 mg/kg bw/day following prolonged periods of markedly reduced food consumption and body weight losses (Table 4). Four and 3 does at 250 and 500 mg/kg bw/day, respectively, were sacrificed in extremis due to marked decrease in food consumption and body weight loss prior to scheduled sacrifice. There was no mortality at 25 mg/kg bw/day. In addition, one animal in the control group and another one in the high dose group were accidentally killed by intubation injury.

Table 4. Summary of maternal mortality data (n=22)

Dose (mg/kg/day)	0	25	100	250	500
Number sacrificed in extremis	0	0	2	4	3
Number killed accidentally by intubation injuries	1	0	0	0	1

Data excerpted from pages 28-29 of the study report.

3. Body weight, body weight gain, and food consumption (Table 5)

Test substance-related reductions in maternal body weight parameters were observed at 100 mg/kg bw/day and above. There were no test substance-related effects on body weight parameters at 25 mg/kg bw/day. Body weight gains that were lower or statistically significantly lower than controls were evident after about a week of dosing resulting in overall body weight gains calculated over Day 7 to 29G that were 35%, 78%, and 75% lower than control at 100, 250, and 500 mg/kg bw/day, respectively. Maternal body weight changes using the final body weight adjusted for the weight of the gravid uterus also reflected greater losses when compared with the control group; during Day 7 to 29G, does lost an average of 181, 291, and 267 grams at 100, 250, and 500 mg/kg bw/day, respectively, compared with an average loss of 107 and 88 grams at 0 and 25 mg/kg bw/day, respectively.

Test substance-related effects on maternal food consumption were observed at 100 mg/kg bw/day and above (Table 5). Reductions in mean food consumption were evident after the first few days of dosing and persisted for the duration of the study, and statistically significant reductions in group means were observed on many occasions during the study at 250 mg/kg bw/day and above. Overall food consumption means calculated from gestation day 7 to 29 were approximately 9%, 20%, and 20% lower than the control group mean at 100, 250, and 500 mg/kg bw/day, respectively. At 25 mg/kg bw/day, there was no test substance-related effect on maternal food consumption.

Table 5. Maternal body weight, body weight gain, and food consumption

Parameter	Dose (mg/kg bw/d)				
	0	25	100	250	500
Body weight day 29 (g)	3579±198	3591±252	3496±254	3315±362* (↓7%)	3357±266 (↓6%)
Adjusted[a] body wt day 29 (g)	3101±155	3139±189	3073±233	2942±266	2991±192
Body weight gain 7–29G (g)	368±99	364±100	241±186 (↓35%)	81±341* (↓78%)	91±228* (↓75%)
Adjusted[a] body weight gain 7–29G (g)	-107±71	-88±100	-181±165 (↓69%)	-291±239 (↓167%)	-267±139 (↓150%)
Food consumption 7–29G (g/day)	121±7	121±6	110±15* (↓9%)	97±27* (↓20%)	97±21* (↓20%)

[a] Terminal body weight minus the gravid uterus weight.

* Statistically significant differences at $p<0.05$

Data in this table obtained from pages 30 – 38 of the study report.

4. Reproductive outcome (Cesarean section data)

Prior to scheduled sacrifice (GD 29), 4 does at 250 mg/kg bw/day and 3 does at 500 mg/kg bw/day aborted or delivered early (Table 6). The individual animal data indicated that all but one 250 mg/kg bw/day female was observed with diarrhea and/or reduced or absent of feces prior to signs of abortion. In general, these females that aborted or delivered on the day of scheduled termination had prolonged periods of markedly reduced food consumption and body weight losses that preceded the abortion or early delivery. Based on these marked reductions in body weight and food consumption and associated observations of diarrhea and reduced or absent of feces, these late gestation abortions might be influenced by the maternal toxicity as suggested by the data shown in Table 6.

Data for the mean numbers of implantations, resorptions, live and dead foetuses, and litter sex ratio were comparable across all groups tested (Table 7).

Table 6. Summary data on number of does aborted or delivered early

Animal No.	Dose (mg/kg)	Sacrificed in extremis	Gestation day aborted or delivered early	Body weight loss (gm)	Gestation days with marked decrease in food consumption
405	250	Yes	25	322	12-25
410	250	Yes	29	688	14-29
411	250	Yes	26	392	15-26
413	250	Yes	22	399	12-22
506	500	Yes	27	335	21-27
516	500	Yes	29	166	13-14 & 21-27
518	500	Yes	29	520	15-17 & 25-29

Data excerpted from page 20 of the report.

Table 7. Cesarean section observations

Parameter	Dose (mg/kg bw/d)				
	0	**25**	**100**	**250**	**500**
Number in each group	22	22	22	22	22
Number pregnant	18	22	21	22	21
Number aborted/delivery early	0	0	0	4	3
Number aborted	0	0	0	0	1
Number with live fetous at scheduled termination	18	22	19	18	17
Number with total resorptions	0	0	0	0	0
Mean corpora lutea	9.5±2.3	9.0±2.4	8.8±1.6	8.9±1.4	8.1±2.2
Mean implants/Litter	8.9±1.8	7.9±1.7	7.7±1.7	7.7±1.5	7.6±2.1
Total resorptions/Litter	0.33±0.59	0.36±0.58	0.21±0.42	0.33±0.59	0.18±0.39
Early resorption/litter	0.28±46	0.14±0.47	0.16±0.17	0.11±0.32	0.00
Late resorption/litter	0.06±0.24	0.23±0.43	0.05±0.23	0.22±0.55	0.18±0.39
Mean live fetuses/Litter	8.6±1.7	7.5±1.8	7.5±1.7	7.4±1.6	7.4±2.1
Mean dead fetuses/Litter	0	0	0	0	0
Fetal sex ratio[b]	0.48±0.16	0.49±0.17	0.46±0.17	0.46±015	0.52±0.19
Mean fetal weight (g)	40.1±3.0	40.6±4.1	39.9±5.6	37.5±6.0* (↓6%)	37.7±5.4* (↓6%)

[a] One control was accidentally killed as a result of intubation injuries.
[b] Number of male foetuses/total foetuses per litter
* Statistically significant differences at $p < 0.05$. Data excerpted from pages 41 – 42 of the study report.

5. Gross pathology

No test substance-related gross lesions were observed at necropsy. At 25 mg/kg bw/day, one female had a discoloured spleen; since this was observed in a single animal, it was not considered test substance-related. At 100 mg/kg bw/day, two females that were sacrificed early due to weight loss and markedly reduced food consumption were observed with empty intestines. At 250 and 500 mg/kg bw/day, there were observations in one or two animals involving the abdominal viscera and included empty intestines and discoloured organs; these observations were most frequently observed in animals that had either aborted or delivered early.

B. FETAL EFFECTS

Test substance-related and statistically significant reductions in mean fetal weight were observed at 250 and 500 mg/kg bw/day (Table 7). Mean fetal weights were approximately

6% lower than the control group mean at 250 and 500 mg/kg bw/day.

There were no test substance-related fetal malformations or variations observed at any dose level tested (Table8). The incidence of foetal external, visceral, head, or skeletal malformations or variations were comparable across all dose groups.

Table 8. Fetal alterations

Parameter	Dose (mg/kg bw/d)				
	0	**25**	**100**	**250**	**500**
No. Examined (external/skeletal)	154 (18)	166 (22)	142 (19)	139 (19[a])	134 (18[b])
No. Examined (visceral/head)	154 (18)	166 (22)	142 (19)	139 (19[a])	134 (18[b])
Malformations					
Umbilicus, hernia			1 (1)		
Gastroschisis			1 (1)		
Open eye	1 (1)				
Persistent truncus arteriosus	1 (1)		1 (1)		
Heart cor triloculare			1 (1)		
Misshapen A-V valve	1(1)				
Enlarged aortic valve	1 (1)				
Absent spleen			1 (1)		
Malpositioned adrenal gland			1 (1)		
Enlarged liver			1 (1)		
Malpositioned kidney			1(1)		
Bent Hyoid	1 (1)	1 (1)		3 (1)	1 (1)
Fused thoracic arch			1 (1)		
Absent rib			1 (1)		
Fused rib			1 (1)		
Variations					
Limb hyperflexion				1 (1)	
Small pancrease			1 (1)		
Small gallbladder			2(2)	2(1)	2 (2)
Incomplete ossification, pubis				6 (1)	
Misaligned thoracic centrum			1 (1)		
Branched rib			1 (1)		
Unossified sternebrae	5 (4)	16 (6)	10 (7)	9 (5)	2 (2)
Misaligned sternebrae			1 (1)		
Fused sternebrae	3(5)	2 (3)	4 (2)		

Note: Number examined and affected are presented as foetuses (litters). Statistical analyses are only conducted on the individual endpoints; the overall totals are presented for information only.

There were no statistically significant differences (mean percent affected per litter) from control at $p < 0.05$.

a Doe #410 delivered early on DG 29 just prior to scheduled sacrifice; the foetuses were intact and, therefore, examined for external, visceral, head, and skeletal deviations.

b Doe #518 delivered early on DG 29 just prior to scheduled sacrifice; the foetuses were intact and, therefore, examined for external, visceral, head, and skeletal deviations.

Data in this table obtained from pages 43 – 48 of the study report.

III. CONCLUSION

Under the conditions of this developmental oral toxicity study in rabbit, cyantraniliprole at dose levels of 100, 250, and 500 mg/kg/day produced maternal effects consisting of the following: increased incidence of diarrhea, decreased body weight, body weight gains, decreased food consumption, increased observations of reduced or absent of feces, and increased number of death or number of animal sacrificed in extremis. At 250 and 500 mg/kg bw/day, there was a treatment related decrease in fetal body weights.

The maternal no-observed-adverse-effect level (NOAEL) was 25 mg/kg bw/day. **The LOAEL was 100 mg/kg bw/day based on mortality, increased clinical signs of toxicity (diarrhoea and reduced or absent of feces), decreased body weights, and reduced food consumption.**

The developmental NOAEL was 100 mg/kg bw/day. **The developmental LOAEL was 250 mg/kg bw/day, based on reductions in mean fetal weight.**

This study is fully reliable (acceptable/guideline) and satisfies the requirement for a developmental toxicity study in rabbits (OPPTS 870.3700; OECD (Part 414); Directive 87/302/ECC 87/302/EEC, Part B; MAFF 12 Nousan 8147).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.3.7/01

Report: Lowe, C. (2009); Cyantraniliprole (DPX-HGW86) technical: 28 day repeat dermal application study in rats. Eurofins, Product Safety Laboratories, Dayton, New Jersey, USA. Laboratory No.: 25531. DuPont Report No.**:** DuPont-21316, Revision No.1. Study completion: April 7, 2009. Study amended: April 23, 2009. MRID 48119970. Unpublished.

Guidelines: U.S. EPA OPPTS 870.3200 (1998)
OECD Part 410 (1981)
JMAFF Guideline 2-1-10 Notification 12 Nousan (2000)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 28-day dermal toxicity study (MRID 48119970), cyantraniliprole (94.5%; HGW86-230) was applied to the shaved, intact dorsal skin of male and female Hsd:Sprague Dawley® rats (10/sex/dose). The test substance was moistened with distilled water and applied for 29 daily (consecutive) applications. The rats were exposed to the test substance for 6 hours per day at 0, 100, 300, and 1000 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, ophthalmology, organ weights, and gross and microscopic pathology. Dermal irritation was conducted weekly on days 4, 11, 18, 25, and at study termination (day 29/30) according to the Draize *et al*., 1944.

Under the conditions of this study cyantraniliprole treatment did not affect survival, body weights, nutritional parameters, ophthalmology, organ weights, or clinical or anatomic pathology parameters.

Slight or mild erythema was observed at the application site of male and female rats in all test substance treated groups; the occurrence was less frequent in females than in males. Dermal irritation was considered to be treatment related and a local effect.

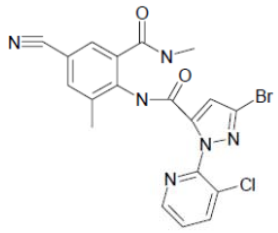
The no-observed-adverse-effect level (NOAEL) for systemic effects was 1000 mg/kg bw/day (highest dose tested) for males and females. No LOAEL was established because no adverse systemic toxicity was seen at any dose levels. The highest dose was the limit dose for dermal toxicity study.

This study is fully reliable (acceptable/guideline) and meets the data requirements for a 28-day dermal toxicity study (U.S. EPA OPPTS 870.3200; OECD Part 410; JMAFF Guideline 2-1-10 Notification 12 Nousan).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Off-white solid
CAS #	736994-63-1
Stability of test compound:	Not determined. The test substance was applied promptly to the skin after moistening with distilled water

2. Vehicle and/or positive control: distilled water
3. Test animals

Species:	Rat
Strain:	Hsd:Sprague Dawley®
Age at dosing:	Approximately 9 weeks
Weight at dosing:	254–287 g for males; 170–204 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	7 days
Diet:	Purina LabDiet Certified Rodent Meal #5002M, *ad libitum*.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	20–24°C
Humidity:	57–78%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

   July 29, 2008 to 28-August 28, 2008

2. Animal assignment and treatment

   Doses of 0, 100, 300, and 1000 mg/kg bw/day were selected for this study. The 1000 mg/kg bw/day dosage is a limit dose level. The other dosages were selected to establish a no-observed-adverse-effect level (NOAEL) and to assess a dose response for any observed effects.

   Four groups of 10 animals/sex/dosage were administered dermal doses of either cyantraniliprole or the vehicle daily for 29 days (Table 1). Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study

Group No.	No./Sex/Group	Daily Dermal Dose mg/kg bw
1	10	0 (control)
2	10	100
3	10	300
4	10	1000

3. Dose preparation and analysis

   The test substance was moistened with vehicle to form a thick paste. Control animals received vehicle alone. The test substance was weighed out for each animal based on the most recently determined body weight. Based on this information, it can be concluded that the animals received the targeted dose of test substance during the study.

4. Preparation and treatment of animal skin

   On the day prior to the first application, the fur of each animal was closely clipped and checked for any abnormalities. A dose of cyantraniliprole, moistened with distilled water to form a thick paste, was applied to the intact skin of each animal. The approximate total body surface area covered for the 100, 300, and 1000 mg/kg bw/day groups was 0.4%, 1.7%, and 10% (males)/6.7% (females), respectively. It was not possible to cover an area of 10% of the total body surface for the test groups without excessively diluting the test substance. The test animals were dosed for 6 hours. Following the 6 hours exposure period, the wrappings were removed, and the application site of each test animal was wiped with a 3% soap solution (Ivory dishwashing liquid) and then rinsed with tap water. The application site was patted dry and examined for any skin irritation.

5. Statistics

Group means and standard deviations were calculated for body weight, daily body weight gain, daily food consumption, daily food efficiency, organ weight, and organ-to-body/brain weight ratio data. Data within groups were evaluated for homogeneity of variances and normality by Bartlett's test. Where Bartlett's test indicated homogeneous variances, treated and control groups were compared using a One-Way Analysis of Variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's t-test for multiple comparisons. Where variances were considered significantly different by Bartlett's test, groups were compared using a non-parametric method (Kruskal-Wallis non-parametric analysis of variance followed by Dunn's test) (INSTAT Biostatistics, Graph Pad Software, San Diego, CA). Differences among groups were judged to be significant at a probability value of $p < 0.05$. Male and female rats were evaluated separately.

Means and standard deviations were calculated for clinical pathology quantitative data. Data within groups were initially analyzed using Levene's test for variance homogeneity (Levene, H., 1960), and the Shapiro-Wilk test for normality (Shapiro, S.S. and Wilk, M.B., 1965). If variances were considered to be not significantly different, groups were compared using a One-Way Analysis of Variance (ANOVA) (Snedecor, G.W. and Cochran, W.G., 1967) followed by Dunnett's t-test for multiple comparisons (Dunnett, C.W., 1964, 1980, Tamhane, A.C., 1979). If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. Where variances were considered significantly different by Levene's test, groups were compared using a non-parametric method (Kruskal-Wallis non parametric analysis of variance followed by Dunn's test) (Kruskal, W.H. and Wallis, W.A., 1952, Dunn, O.J., 1964). Differences among groups were judged to be significant at a probability value of $p < 0.05$. Male and female rats were evaluated separately.

C. METHODS

1. Observations

Animals were observed twice daily for mortality, and signs of illness, injury, or abnormal behaviour, and examined daily for clinical signs of toxicity. Detailed clinical observations were done on the first day of dosing and weekly thereafter.

2. Body weights

All animals were weighed once per week.

3. Food consumption and food efficiency

Food consumption was recorded for each animal over the weighing interval. Food efficiency and was calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, coagulation)

Blood samples were collected from all animals approximately 4 weeks after initiation of the study. Animals were fasted overnight prior to sample collection. At sacrifice, blood and bone marrow were collected. Animals were evaluated for haematology, clinical chemistry, and coagulation. The parameters evaluated were the following:

Hematology and coagulation

red blood cell count	Red cell distribution width
hemoglobin	absolute reticulocyte count
hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular(cell)hemoglobin concentration	
microscopic blood smear examination	
prothrombin time activated partial	thromboplastin time

Clinical chemistry parameters

aspartate aminotransferase	total protein
alanine aminotransferase	albumin
sorbitol dehydrogenase	globulin
alkaline phosphatase	calcium
total bilirubin	inorganic phosphorus
urea nitrogen	sodium
creatinine	potassium
cholesterol	chloride
triglycerides	glucose

6. Sacrifice and pathology

At termination, animals were sacrificed by exsanguination from the abdominal aorta under isoflurane anesthesia. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 2. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (1000 mg/kg bw/day) and control (0 mg/kg bw/day) were processed to slides and evaluated microscopically (Table 2).

Table 2. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Brain	X	X
Spleen	X	X
Heart	X	X
Liver	X	X
Kidneys	X	X
Adrenal glands	X	X
Thymus	X	X
Testes	X	X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted[a]
Epididymides	X	X
Prostate + seminal vesicles		X
Esophagus		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Trachea		X
Larynx		X
Pharynx		X
Nasal turbinates		X
Ovaries with oviducts	X	X
Manibular lymph node		X
Mesenteric lymph node		X
Uterus including fallopian tubes	X	X
Vagina		X
Mammary glands (females)		X
Stomach		X
Pituitary		X
Thyroid gland		X
Parathyroid gland		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur		X
Sternum with bone marrow		X
Aorta		X
Eyes including retina and optic nerve		X
Urinary bladder		X
Skin (treated and untreated)		X
Gross observations		X

[a] Only tissues from animals in the highest dose and control groups were evaluated

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs of systemic toxicity were observed.

2. Dermal irritation

Slight erythema was observed at the dose site of most male rats treated with the test substance on examination day 18 (Table 3). The increase incidence did not show a dose-related response. Well-defined erythema was observed at the dose site of one male rat in the 100 mg/kg bw/day group while another male rat in the same dose group

exhibited oedema. No erythema or oedema was observed in male control rats. In females, the incidence of erythema was fewer than in the male rats. One female control rat exhibited slight erythema. No edema was observed in female rats.

Table 3. Incidence of dermal irritation-erythema

mg/kg bw/day	0	100	300	1000
Males				
Day 4	0/10	0/10	0/10	2/10
Day 18	0/10	8/10	8/10	7/10
Day 29	0/10	2/10	5/10	2/10
Females				
Day 4	0/10	0/10	1/10	2/10
Day 18	1/10	1/10	2/10	1/10
Day 29	0/10	1/10	2/10	2/10

Data excerpted from page 34 of the report.

3. Mortality

No deaths occurred.

B. BODY WEIGHT AND BODY WEIGHT GAIN

The body weights of controls and the compound treated animals were comparable.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

No test substance-related effect was observed on mean overall food consumption or food efficiency (test days 1 to 29) in any male or female group.

D. OPHTHALMOLOGICAL EXAMINATIONS

No ophthalmological changes were noted in male or female rats at any dose level.

E. CLINICAL PATHOLOGY

1. Hematology

There were no treatment-related changes in group mean hematology parameters in male or female rats.

Red cell distribution width was minimally lower in the 300 mg/kg bw/day females but was considered unrelated to treatment because the change did not show a dose-related response (Table 4).

Table 4: Selected hematology parameters

Dose (mg/kg bw/day)	0	100	300	1000
	Males			

Red Cell Distribution Width (%)	11.8±0.2	11.7±0.3	11.7±0.3	12.0±0.5
	Females			
Red Cell Distribution Width (%)	12.5±1.4	11.7±1.1	11.2*±0.3	12.0±0.5

Data taken from pages 35 and 37 of the study report
*statistically significant from control at p<0.05

2. Clinical chemistry

There were no adverse changes in group mean clinical chemistry parameters in treated male or female rats.

Bilirubin was minimally decreased in female rats dose with 100, 300, and 1000 mg/kg bw/day (86%, 86%, and 71%, respectively). This decrease did not demonstrate a dose-related response and is not considered to be treatment-related.

Table 5. Selective clinical chemistry parameter in female rats: bilirubin

Mg/kg/day	0	100	300	1000
Total bilirubin (mg/dL)	0.14±0.01	0.12±0.01*	0.12±0.01*	0.10±0.01*

* Statistically significantly different from control (p< 0.05)
Data excerpted from page 41 of the study report.

3. Coagulation

There were no treatment-related or statistically significant changes on coagulation parameters in male or female rats.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

In males, a minimal but statistically significant increase in mean absolute and relative adrenal weights (% body and brain weight) was observed in the 1000 mg/kg bw/day group (Table 4). Individual adrenal weights for most animals in this group were within the control group range, and no correlative microscopic pathology was present in any animals in this group. Similar adrenal weight changes were not present in treated female rats. Thymus weight was also slightly decreased in all treated groups relative to the controls; the decrease in the lowest dose group (100 mg/kg bw) showed a statistically significance. However, this slight reduction did not demonstrate a dose related response, and it was not seen in females. The thymus weight change was unlikely to be related to treatment.

Table 5. Selective Organ Weights (gm)				
mg/kg bw/day	**0**	**100**	**300**	**1000**
Males				

Adrenal (paired) Absolut	0.063±0.009	0.068±0.007	0.069±0.005	0.073±0.009*
Relative to bw wt (%)	0.019	0.021	0.022	0.023**
Relative to brain wt (%)	3.30	3.60	3.60	3.80
Thymus: Absolute	0.531±0.071	0.428±0.050**	0.464±0.032	0.463±0.099
Relative to bw wt (%)	0.162	0.134*	0.146	0.146
Relative to brain wt (%)	27.6	22.6	24.1	24.2
Females				
Adrenal (paired): absolute	0.085±0.013	0.089±0.008	0.081±0.006	0.089±0.013
Relative to bw wt (%)	0.040	0.042	0.039	0.042
Relative to brain wt (%)	4.90	4.90	4.90	5.00
Thymus: Absolute	0.320±0.072	0.378±0.054	0.369±0.054	0.328±0.069
Relative to bw wt (%)	0.151	0.176	0.176	0.154
Relative to brain wt (%)	18.3	20.7	20.8	18.5

**: Statistically significant ($p<0.01$) by Kruskal-Wallis test.
*: Statistically significant ($p<0.05$) by Dunnett's Multiple Comparisons test.
Data excerpted from pages 42-43 of the report.

2. Gross pathology and histopathology

No test substance-related gross or microscopic lesions were found in any dosed groups relative to the controls.

III. CONCLUSION

Dermal application of cyantraniliprole at dose levels up to 1000 mg/kg did not produce treatment-related effects on following parameters: survival, clinical signs of toxicity, body weights, food consumption, clinical pathology, hematology, organ weights, gross observation, and hispathology. The no-observed-adverse-effect level (NOAEL) for systemic effects was 1000 mg/kg bw/day (highest dose tested and limit dose) for males and females. No LOAEL was established. It should be noted that microvesiculation of adrenal cortex was not found in this 28-day dermal toxicity study.

This study is fully reliable (acceptable/guideline) and meets the data requirements for a 28-day dermal toxicity study (U.S. EPA OPPTS 870.3200; OECD Part 410; JMAFF Guideline 2-1-10 Notification 12 Nousan).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.10/01

Report: Hoban, D. (2009). Cyantraniliprole (DPX-HGW86) Technical: 28-Day Immunotoxicity Feeding Study in Rats. DuPont Haskell Laboratories, Newark, Delaware 19714, USA. Laboratory Report No.: DuPont-21467. 10 April 2009. MRID 48119971. Unpublished.

Guidelines: OECD None
OPPTS 870.7800 (1998)

Deviations: None

GLP: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive Summary:

In an immunotoxicity study (MRID 48119971), cyantraniliprole technical (94.5% a.i. batch# HGW86-0603-1) was administered to 10 Crl:CD®(SD) rats/concentration/sex at concentrations of 0, 20, 200, 2000, or 20000 ppm (mean daily intakes for males/females were: 0/0, 1.7/1.8 , 17/18, 166/172, or 1699/1703 mg/kg bw/day); males were treated for 28 days and females were treated for 29 days. The positive control group consisting of 5 Crl:CD®(SD) rats/sex were administered cyclophosphamide monohydrate (25 mg/kg bw/day) intraperitoneally for the 6 days prior to sacrifice.

On Day 22 (males) and Day 23 (females), the test animals were immunized with an injection of sheep red blood cells (SRBC). On Day 28 (males) and Day 29 (females), blood samples were collected from the test animals. The serum samples were assayed for their concentration of SRBC-specific IgM antibodies to provide a quantitative assessment of humoral immune response. Additional parameters that were evaluated include mortality, clinical signs of toxicity, body weight, body weight gain, food consumption, food efficiency, organ weights (i.e. brain, spleen, and thymus), and macroscopic pathology.

In the treated animals there were no unscheduled mortalities in any treatment group; there were no treatment-related effects on body weight, body weight gain, food consumption, food efficiency, or organ weights (i.e. spleen, thymus, and brain); and there were no treatment related findings for clinical signs of toxicity or macroscopic pathology.

The systemic toxicity NOAEL is the HDT at 20000 ppm (M/F: 1699/1703 mg/kg/day). The LOAEL is >20000 ppm.

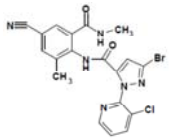
The SRBC-specific IgM ELISA results did not indicate any treatment-related immunosuppressive effects. There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response.

The immunotoxicity NOAEL is the HDT at 20000 ppm (M/F: 1699/1703 mg/kg/day). The LOAEL is >20000 ppm.
This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical (DPX-HGW86-230)
   Description: Off-White Solid
   Lot/Batch #: HGW86-0603-1
   Purity: 94.5% a.i.
   Compound Stability: Stable at ambient temperatures
   CAS#: 736994-63-1
   Structure:



2. Vehicle and Positive Control
   Vehicle: Treated and Control Animals: Basal Diet
   Positive Control: Cyclophosphamide Monohydrate (Source and Batch No.: Not provided)

3. Test Animals:

Species:	Rat	
Strain:	Crl:CD®(SD)	
At Study Initiation:	Age:	~6 weeks old
	Weight:	M: 157.9-214.1 g / F: 136.8-182.0 g
Source:	Charles River Laboratories, Inc. [Raleigh, NC, USA]	
Housing:	Individually in suspended, stainless steel, wire-mesh cages	
Diet:	Certified Rodent LabDiet® *ad libitum* (PMI® Nutrition International, LLC)	
Water:	Tap water *ad libitum*	
Environmental Conditions:	Temperature:	18-26ºC
	Humidity:	30-70%
	Air Changes:	Information was not provided
	Photoperiod:	12 hrs dark / 12 hrs light
Acclimation Period:	7 days	

B. STUDY DESIGN:

1. Experimental Dates
   18 August 2008 – 10 April 2009

2. Animal Assignment

Animals were assigned by computerized randomization to the test groups noted in Table 1. Dietary concentrations were selected based on the results of a concurrent 2-year feeding study in rats.

Table 1: Study Design[a]

Test Group	Dietary Concentration (ppm)	Dose (mg/kg/day)		Number of Animals	
		Male	Female	Male	Female
Control	0	0	0	10	10
Low	20	1.7	1.8	10	10
Mid-Low	200	17	18	10	10
Mid-High	2000	166	172	10	10
High	20000	1699	1703	10	10
Positive Control[b]	-	-	-	5	5

[a] Information was obtained from page 12 of the study report
[b] Intraperitoneally dosed with cyclophosphamide (25 mg/kg/day) for 6 days in males (Days 22-28) and females (Days 23-29)

3. Diet Preparation and Analysis

The test substance was added directly to rodent diet and thoroughly mixed for a period of time that was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. Samples were analyzed to verify concentration and homogeneity of cyantraniliprole in the diets. The analysis results show that the test substance was homogeneously mixed and at the targeted concentrations for all dietary levels. Stability of cyantraniliprole from 20 to 20000 ppm in the diets was established in a concurrent study. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

Table 2: Results of Diet Analysis[a]

Parameter	Result
Homogeneity (%RSD)	1-5%
Stability	Stability data was not provided[b]
Concentration (% nominal concentration)	90.0-100.5%

[a] Information was obtained from pages 20 and 58 of the study report
[b] The study cited is:
DPX-HGW86 Technical: Combined Chronic Toxicity/Oncogenicity Study 2-Year Feeding Study in Rats. Korea Institute of Toxicology. Laboratory Study Number: DuPont-19181. Unpublished

4. Statistics

Significance was judged at $p<0.05$. Separate analyses were performed on the data for each sex.

Table 3: Statistical Analysis[a]

Parameter	Statistical Algorithm
Body weight Body weight gain Food consumption Food efficiency Humoral immune function data[b] Organ weights	If Levene's test for homogeneity and Shapiro-Wilk test for normality are significant Then use Kruskal-Wallis test Dunn's test Else use One-way analysis of variance Dunnett's test

[a] Information was obtained from page 17 of the study report
[b] SRBC-specific serum IgM antibody titer data was transformed to Log_2 to obtain normality or homogenous variances. Statistical comparison was not performed for the positive control.

C. METHODS

1. Observations

   Cage-side examinations to detect moribund or dead rats and abnormal behavior and/or appearance among rats were conducted at least once daily throughout the study.

2. Body weights
   During the test period, all animals were weighed once per week.

3. Food consumption, Food efficiency, and Daily intake
   Food consumption was recorded for each animal over the weighing interval; the amount of food consumed was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Humoral immune function, Gross pathology and Organ weights
   On Day 22 (males) or 23 (females), animals were immunized via intravenous injection into the lateral tail vein with 0.5 mL of 4×10^8 SRBC/mL (Lampire Biological Laboratories [Pipersville, PA, USA]). Following the IV injection of SRBC, rats in the positive control group were injected intraperitoneally once a day for 6 days with 25 mg/kg/day of the known immunosuppressive agent, cyclophosphamide monohydrate in deionized water, at a dose volume of 10 mL/kg body weight. On Day 28 (males) or 29 (females), the animals were euthanized by carbon dioxide anesthesia and exsanguination. Approximately 4 mL of blood was collected at the sacrifice from the abdominal vena cava, while the animal was under carbon dioxide anesthesia. The blood from each rat was processed to serum and frozen at ≤-60°C until analyzed. Spleen, thymus and brain from treated groups were weighed and discarded without further evaluation. Humoral immune function was evaluated by examining serum from individual test substance treated animals for SRBC-specific IgM levels with an enzyme-linked immunosorbent assay (ELISA). The serum from each animal was assayed as 10 serial, 2-fold dilutions, with 1 replicate per dilution. The optical density (OD) of the serum samples was measured at the 405 nm wavelength with a Molecular Devices microplate spectrophotometer. SRBC-specific serum IgM titer data were analyzed with SoftMax Pro software version 4.3.1 LS. For each serum sample, a semi-log graph of the data was created and the linear portion of the curve was identified by using a log-log curve fit. A slope of not less than -0.600, but not more than -1.200 was obtained. The serum dilution expected to produce an OD of 0.5 was determined by using Microsoft Excel® using 3 OD readings, one point above and one below approximately 0.5. The "titer" of each animal was defined as the reciprocal of the serum dilution that had a value of 0.5. If a serum was tested and no points had an OD value of greater than or equal to 0.5, the reciprocal of the starting dilution closest to an OD value of 0.5 was used as the titer.

   Pooled male and female serum collected from rats injected with cyclophosphamide monohydrate was run concurrently with the study samples as a positive control. The pooled samples consisted of equal aliquots of serum taken from either the male or female rats dosed with cyclophosphamide monohydrate.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical Signs of Toxicity There were no treatment-related findings observed.
2. Mortality There were no unscheduled mortalities.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no treatment-related effects observed.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no treatment-related effects observed.

D. HUMORAL IMMUNE FUNCTION, GROSS PATHOLOGY, AND ORGAN WEIGHTS

1. Humoral Immune Function

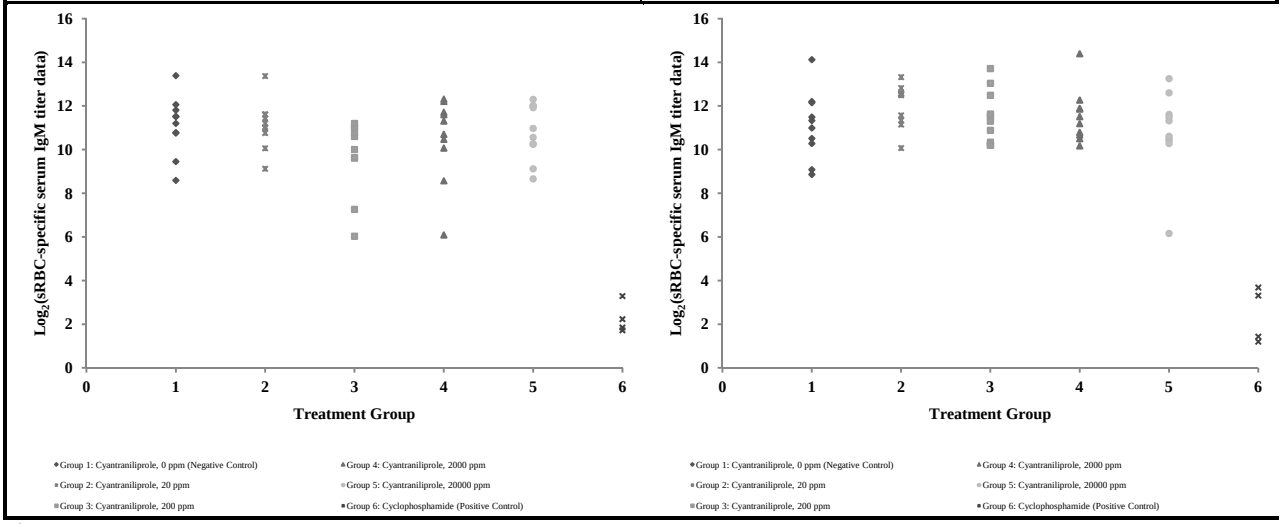
The results of the SRBC-specific IgM ELISA did not indicate any treatment-related immunosuppressive effects (Table 4). There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response (Figure 1). The positive control group demonstrated a statistical decrease in antibody concentration as compared to the vehicle controls.

Table 4: SRBC-Specific IgM ELISA Results[a] (n=10)

	Mean Log_2(SRBC-specific serum IgM titer data) ± SD (% difference from negative control)	
Dose Group (ppm)	**Males**	**Females**
Negative Control (0)[b]	11.11 ± 1.34	11.10 ± 1.55
20[b]	11.12 ± 1.10 (0.1)	12.04 ± 0.98 (8.5)
200[b]	9.71 ± 1.73 (-12.6)	11.53 ± 1.22 (3.9)
2000[b]	10.50 ± 1.91 (-5.5)	11.52 ± 1.21 (3.8)
20000[b]	10.81 ± 1.27 (-2.7)	10.82 ± 1.91 (-2.5)
Positive Control[c]	2.27 ± 0.71 (-79.6) [n=4]	2.41 ± 1.27 (-78.3) [n=4]

n = 10 animals per dose group unless otherwise noted
[a] Information was obtained from pages 36 and 37 of the study report
[b] SRBC-specific serum IgM titer data for each animal
[c] SRBC-specific serum IgM titer data for pooled samples

Figure 1: Individual Primary Immune Response Data[a, b]



[a] Figures were created by the reviewer with information obtained from pages 90, 91, and 94 of the study report
[b] Treated groups: SRBC-specific serum IgM titer data for each animal
Positive Control Groups: SRBC-specific serum IgM titer data for pooled samples

2. Gross Pathology
There were no treatment-related findings observed.

3. Organ Weights
There were no test substance-related effects observed. Decreased thymus weights in males at the 200 ppm and 20000 ppm doses were not dose-related and were not considered treatment-related (Table 5).

Table 5: Organ Weight Results[a] (n=10)

Males	Mean Terminal Weight (g) ± SD (% difference from negative control)		
Dose Group (ppm)	Body	Brain	Thymus
0	391.7 ± 34.1	1.950 ± 0.111	0.769 ± 0.134
20	401.2 ± 35.7 (2.4)	1.937 ± 0.034 (-0.7)	0.670 ± 0.125 (-12.9)
200	388.5 ± 18.8 (-0.8)	1.950 ± 0.083 (0.0)	0.598 ± 0.090 (-22.2)*
2000	408.1 ± 24.4 (4.2)	1.942 ± 0.098 (-0.4)	0.705 ± 0.103 (-8.3)
20000	399.2 ± 20.7 (1.9)	1.993 ± 0.119 (2.2)	0.656 ± 0.119 (-14.7)

	Mean Relative Thymus Weight (Parameter) ± SD (% difference from negative control)	
Dose Group (ppm)	% of Body Weight	% of Brain Weight
0	0.197 ± 0.033	39.518 ± 7.192
20	0.167 ± 0.027 (-15.2)	34.667 ± 6.805 (-12.3)
200	0.154 ± 0.020 (-21.8)*	30.569 ± 3.822 (-22.6)*
2000	0.173 ± 0.024 (-12.2)	36.346 ± 5.626 (-8.0)
20000	0.165 ± 0.032 (-16.2)*	33.192 ± 7.258 (-16.0)

n= 10 animals per dose group
[a] Information was obtained from pages 38-40 of the study report
* $p<0.05$

III. CONCLUSION

In the treated animals there were no unscheduled mortalities in any treatment group; there were no treatment-related effects on body weight, body weight gain, food consumption, food

efficiency, or organ weights (i.e. spleen, thymus, and brain); and there were no treatment related findings for clinical signs of toxicity or macroscopic pathology.

The systemic toxicity NOAEL is the HDT at 20000 ppm (M/F: 1699/1703 mg/kg/day). The LOAEL is >20000 ppm.

The SRBC-specific IgM ELISA results did not indicate any treatment-related immunosuppressive effects. There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response.

The immunotoxicity NOAEL is the HDT at 20000 ppm (M/F: 1699/1703 mg/kg/day). The LOAEL is >20000 ppm.

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in rats.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.10/02

Report: Hoban, D. (2011). Cyantraniliprole (DPX-HGW86) Technical: 28-Day Immunotoxicity Feeding Study in Mice. DuPont Haskell Laboratories, Newark, Delaware 19714, USA. Laboratory Report No.: DuPont-21468, Revision No. 1. 11 April 2011. MRID 48119972. Unpublished.

Guidelines: OECD None
OPPTS 870.7800 (1998)

Deviations: None

GLP: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:
In an immunotoxicity study (MRID 48119972), cyantraniliprole technical (94.5% a.i. batch# HGW86-0603-1) was administered to 10 Crl:CD1®(ICR) mice/concentration/sex at concentrations of 0, 20, 150, 1000, or 7000 ppm (mean daily intakes for males/females were: 0/0, 3.0/4.1, 23/32, 154/224, or 1065/1386 mg/kg bw/day); males were treated for 28 days and females were treated for 29 days. The positive control group consisting of 5 Crl:CD1®(ICR) mice/sex were administered cyclophosphamide monohydrate (25 mg/kg bw/day) intraperitoneally for the 5 days prior to sacrifice.

On Day 23 (males) and Day 24 (females), the test animals were immunized with an injection of sheep red blood cells (SRBC). On Day 28 (males) and Day 29 (females), blood samples were collected from the test animals. The serum samples were assayed for their concentration of SRBC-specific IgM antibodies to provide a quantitative assessment of humoral immune response. Additional parameters that were evaluated include mortality, clinical signs of toxicity, body weight, body weight gain, food consumption, food efficiency, organ weights (i.e. brain, spleen, and thymus), and macroscopic pathology.

In the treated animals there were no unscheduled mortalities in any treatment group; there were no treatment-related effects on body weight, body weight gain, food consumption, or food efficiency; there were no dose-related effects on organ weights (i.e. spleen, thymus, and brain); and there were no treatment related findings for clinical signs of toxicity or macroscopic pathology.

The systemic toxicity NOAEL is the HDT at 7000 ppm (M/F: 1065/1386 mg/kg/day). The LOAEL is >7000 ppm.

The SRBC-specific IgM ELISA results did not indicate any treatment-related immunosuppressive effects. There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual

animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response.

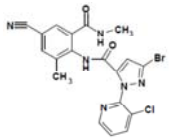
The immunotoxicity NOAEL is the HDT at 7000 ppm (M/F: 1065/1386 mg/kg/day). The LOAEL is >7000 ppm.

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in mice.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical (DPX-HGW86-230)
   Description: Off-White Solid
   Lot/Batch #: HGW86-0603-1
   Purity: 94.5% a.i.
   Compound Stability: Stable at ambient temperatures
   CAS#: 736994-63-1
   Structure:



2. Vehicle and Positive Control
   Vehicle: Treated and Control Animals: Basal Diet
   Positive Control: Cyclophosphamide Monohydrate (Source and Batch No.: Not provided)

3. Test Animals:

Species:	Mouse	
Strain:	Crl:CD1®(ICR)	
At Study Initiation:	Age:	6 weeks old
	Weight:	M: 24.3-31.3 g / F: 18.9-24.1 g
Source:	Charles River Laboratories, Inc. [Raleigh, NC, USA]	
Housing:	Individually in suspended, stainless steel, wire-mesh cages	
Diet:	Certified Rodent LabDiet® *ad libitum* (PMI® Nutrition International, LLC)	
Water:	Tap water *ad libitum*	
Environmental Conditions:	Temperature:	18-26ºC
	Humidity:	30-70%
	Air Changes:	Information was not provided
	Photoperiod:	12 hrs dark / 12 hrs light
Acclimation Period:	6 days	

B. STUDY DESIGN:

1. Experimental
   20 August 2008 – 10 April 2009

2. Animal Assignment

Animals were assigned by computerized, stratified randomization to the test groups noted in Table 1.

Table 1: Study Design[a]

Test Group	Dietary Concentration (ppm)	Dose (mg/kg/day)		Number of Animals	
		Male	Female	Male	Female
Control	0	0	0	10	10
Low	20	3.0	4.1	10	10
Mid-Low	150	23	32	10	10
Mid-High	1000	154	224	10	10
High	7000	1065	1386	10	10
Positive Control[b]	-	-	-	5	5

[a] Information was obtained from page 12 of the study report
[b] Intraperitoneally dosed with cyclophosphamide (25 mg/kg/day) for 5 days in males (Days 23-28) and females (Days 24-29)

3. Diet Preparation and Analysis

The test substance was added directly to rodent diet and thoroughly mixed for a period of time that was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. Samples were analyzed to verify concentration and homogeneity of cyantraniliprole in the diets. The analysis results show that the test substance was homogeneously mixed and at the targeted concentrations for all dietary levels. Stability of cyantraniliprole from 20 to 20000 ppm in the diets was established in a concurrent study. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

Table 2: Results of Diet Analysis[a]

Parameter	Result
Homogeneity (%RSD)	0.6-3%
Stability	Stability data was not provided[b]
Concentration (% nominal concentration)	95.0-103.0%

[a] Information was obtained from pages 21 and 57 of the study report
[b] The study cited is:
DPX-HGW86 Technical: Combined Chronic Toxicity/Oncogenicity Study 2-Year Feeding Study in Rats. Korea Institute of Toxicology. Laboratory Study Number: DuPont-19181. Unpublished

4. Statistics
Significance was judged at $p<0.05$. Separate analyses were performed on the data for each sex.

Table 3: Statistical Analysis[a]

Parameter	Statistical Algorithm
Body weight Body weight gain Food consumption Food efficiency Humoral immune function data[b] Organ weights	If Levene's test for homogeneity and Shapiro-Wilk test for normality are significant Then use Kruskal-Wallis test Dunn's test Else use One-way analysis of variance Dunnett's test

[a] Information was obtained from page 17 of the study report
[b] SRBC-specific serum IgM antibody titer data was transformed to Log_2 to obtain normality or homogenous variances. Statistical comparison was not performed for the positive control.

C. METHODS

1. Observations
   Cage-side examinations to detect moribund or dead mice and abnormal behavior and/or appearance among mice were conducted at least once daily throughout the study.

2. Body weights

   During the test period, all animals were weighed once per week.

3. Food consumption, Food efficiency, and Daily intake
   Food consumption was recorded for each animal over the weighing interval; the amount of food consumed was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Humoral immune function, gross pathology and organ weights
   On Days 23 (males) or 24 (females), animals were immunized via intravenous injection into the lateral tail vein with 0.2 mL of 5×10^8 SRBC/mL (Lampire Biological Laboratories [Pipersville, PA, USA]). Following the IV injection of SRBC, mice in the positive control group were injected intraperitoneally once a day for 5 days with 25 mg/kg/day of the known immunosuppressive agent, cyclophosphamide monohydrate in deionized water, at a dose volume of 10 mL/kg body weight. On Days (males) 28 or 29 (females), the animals were euthanized by carbon dioxide anesthesia and exsanguination. Approximately 1 mL of blood was collected at the sacrifice from the abdominal *vena cava*, while the animal was under carbon dioxide anesthesia. The blood from each mouse was processed to serum and frozen at ≤-60°C until analyzed.

   Humoral immune function was evaluated by examining serum from individual test substance treated animals for SRBC-specific IgM levels with an enzyme-linked immunosorbent assay (ELISA). The serum from each animal was assayed as 10 serial, 2-fold dilutions, with 1 replicate per dilution. The optical density (OD) of the serum samples was measured at the 405 nm wavelength with a Molecular Devices microplate spectrophotometer. SRBC-specific serum IgM titer data were analyzed with SoftMax Pro software version 4.3.1 LS. For each serum sample, a semi-log graph of the data was created and the linear portion of the curve was identified by using a log-log curve fit. A slope of not less than -0.600, but not more than -1.200 was obtained. The serum dilution expected to produce an OD of 0.5 was determined by using Microsoft Excel® using 3 OD readings, one point above and one below approximately 0.5. The “titer” of each animal was defined as the reciprocal of the serum dilution that had a value of 0.5. If a serum was tested and no points had an OD value of greater than or equal to 0.5, the reciprocal of the starting dilution closest to an OD value of 0.5 was used as the titer.

Pooled male and female serum collected from mice injected with cyclophosphamide monohydrate was run concurrently with the study samples as a positive control. The pooled samples consisted of equal aliquots of serum taken from either the male or female mice dosed with cyclophosphamide monohydrate.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical Signs of Toxicity
   There were no treatment-related findings observed.

2. Mortality
   There were no unscheduled mortalities.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no treatment-related effects observed.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no treatment-related effects observed.

D. HUMORAL IMMUNE FUNCTION, GROSS PATHOLOGY, AND ORGAN WEIGHTS

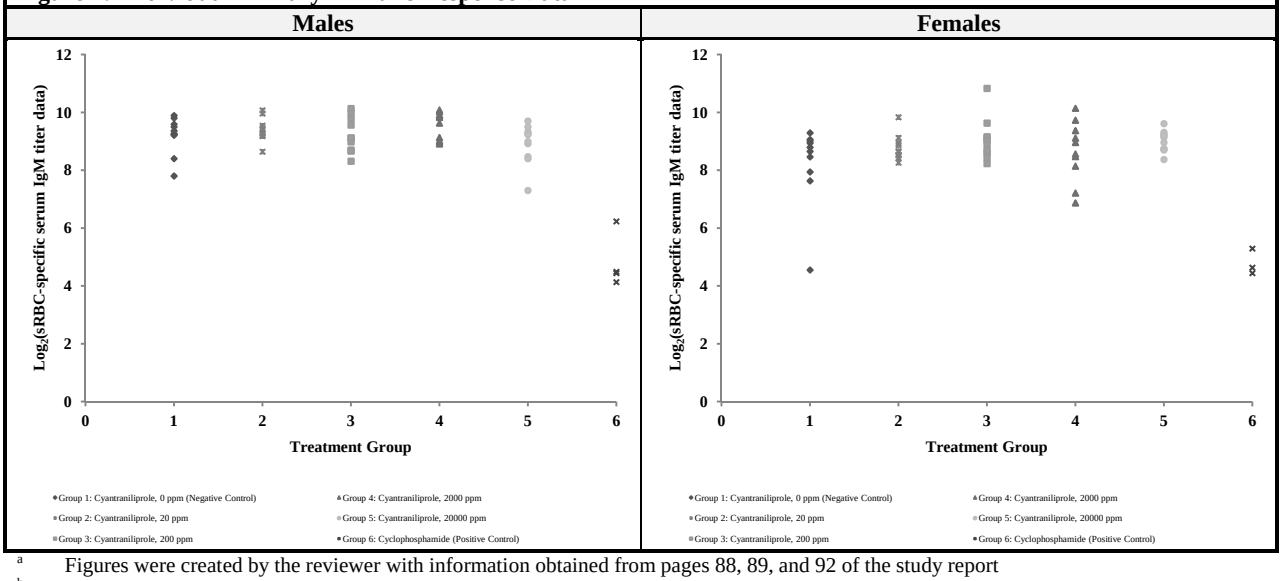
1. HUMORAL IMMUE FUNCTION
   The results of the SRBC-specific IgM ELISA did not indicate any treatment-related immunosuppressive effects (Table 4). There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response (Figure 1). The positive control group demonstrated a statistical decrease in antibody concentration as compared to the vehicle controls.

Table 4: SRBC-Specific IgM ELISA Results[a]

	Mean Log_2(SRBC-specific serum IgM titer data) ± SD (% difference from negative control)	
Dose Group (ppm)	**Males**	**Females**
Negative Control (0)[b]	9.21 ± 0.64	8.23 ± 1.39
20[b]	9.40 ± 0.41 (2.1)	8.78 ± 0.45 (6.7)
150[b]	9.24 ± 0.61 (0.3)	9.06 ± 0.74 (10.1)
1000[b]	9.54 ± 0.48 (3.6)	8.66 ± 1.04 (5.2)
7000[b]	8.91 ± 0.70 (-3.3)	9.00 ± 0.37 (9.4)
Positive Control[c]	4.82 ± 0.95 (-47.7)	4.79 ± 0.45 (-41.8)

n = 10 animals per dose group unless otherwise noted
[a] Information was obtained from pages 35 and 36 of the study report
[b] SRBC-specific serum IgM titer data for each animal
[c] SRBC-specific serum IgM titer data for pooled samples

Figure 1: Individual Primary Immune Response Data[a, b]



[a] Figures were created by the reviewer with information obtained from pages 88, 89, and 92 of the study report
[b] Treated groups: SRBC-specific serum IgM titer data for each animal
Positive Control Groups: SRBC-specific serum IgM titer data for pooled samples

2. Gross Pathology
There were no treatment-related findings observed.

3. Organ Weights
There were no dose-related effects observed. Decreased thymus weights were observed in all dose groups only in males but were not dose-related and were not considered treatment-related (Table 5). No changes in spleen weights were observed for either sex in this study.

Table 5: Organ Weight Results[a]

Males	**Mean Terminal Weight (g) ± SD (% difference from negative control)**		
Dose Group (ppm)	**Body**	**Brain**	**Thymus**
0	35.1 ± 1.7	0.462 ± 0.040	0.067 ± 0.015
20	35.9 ± 2.4 (2.3)	0.464 ± 0.024 (0.4)	0.052 ± 0.012 (-22.4)*
150	34.7 ± 3.0 (-1.1)	0.465 ± 0.018 (0.6)	0.048 ± 0.014 (-28.4)*
1000	32.4 ± 2.0 (-7.7)*	0.480 ± 0.025 (3.9)	0.045 ± 0.007 (-32.8)*
7000	34.3 ± 2.2 (-2.3)	0.460 ± 0.014 (-0.4)	0.050 ± 0.011 (-25.4)*
	Mean Relative Thymus Weight (Parameter) ± SD (% difference from negative control)		
Dose Group (ppm)	**% of Body Weight**	**% of Brain Weight**	
0	0.192 ± 0.046	14.536 ± 2.838	
20	0.143 ± 0.031 (-25.5)*	11.146 ± 2.869 (-23.3)*	
150	0.138 ± 0.040 (-28.1)*	10.337 ± 3.158 (-28.9)*	
1000	0.141 ± 0.023 (-26.6)*	9.491 ± 1.378 (-34.7)*	
7000	0.147 ± 0.035 (-23.4)*	10.866 ± 2.177 (-25.2)*	

n= 10 animals per dose group
[a] Information was obtained from pages 37-39 of the study report
* $p<0.05$

III. CONCLUSION

In the treated animals there were no unscheduled mortalities in any treatment group; there were no treatment-related effects on body weight, body weight gain, food consumption, or food

efficiency; there were no dose-related effects on organ weights (i.e. spleen, thymus, and brain); and there were no treatment related findings for clinical signs of toxicity or macroscopic pathology.

The systemic toxicity NOAEL is the HDT at 7000 ppm (M/F: 1065/1386 mg/kg/day). The LOAEL is >7000 ppm.

The SRBC-specific IgM ELISA results did not indicate any treatment-related immunosuppressive effects. There were no statistical differences in quantity of SRBC-specific IgM in any treatment group when compared with the vehicle controls. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of SRBC-specific antibody response.

The immunotoxicity NOAEL is the HDT at 7000 ppm (M/F: 1065/1386 mg/kg/day). The LOAEL is >7000 ppm.

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in mice.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.10.2 Adrenal and thyroid mechanistic studies

IIA 5.10/03

Report: MacKenzie, S.A. (2010a); Cyantraniliprole (DPX-HGW86) technical: Adrenal and thyroid mechanistic: 90-day feeding study in rats. DuPont Haskell Laboratories; Newark, Delaware, USA. Experimental Pathology Laboratories, Inc.; Durham, North Carolina, USA; Laboratory for Advanced Electron and Light Optical Methods (LAELOM), Raleigh, North Carolina, USA. Laboratory Report No.: DuPont-24319. May 20, 2010. MRID 48119973. Unpublished.

Guidelines: In-house method

Deviations: Not applicable

GLP: Yes. Signed statements of GLP, Qualitiy Assurance, and Data confidentiality were included in the report.

Executive summary:

The objective of this study (MRID 48119973) was to evaluate potential mechanisms of thyroid gland changes and to determine the impact on adrenal gland function following exposure to cyantraniliprole technical. In a 90-day rat study on cyantraniliprole (MRID 48119945), an increased in adrenal microvesiculation was observed in the adrenal cortex of male rats exposed to 20000 ppm in the diet. In addition, thyroid weight and follicular cell hypertrophy occurred in male and female rats, but to a somewhat greater extend in females, at sampling times of 28 and 90 days. The results of the 90-day study suggested that male rats were more sensitive to the adrenal microvesiculation effect while female rats show more sensitivity to the thyroid effects. To explore the possible mechanisms of the thyroid effects, 2 groups of approximately 7 weeks old female Crl:CD®(SD) rats (15/sex/group) were fed control diet or diet containing 20000 ppm cyantraniliprole (94.5%; HGW86-230)(1903 mg/kg bw/day) for 29 days. For adrenal gland effects, three groups of similar age male Crl:CD®(SD) rats (10/sex/group) were fed control diet (2 groups) or diet containing 20000 ppm (1230 mg/kg bw/day) cyantraniliprole for 93 days. For evaluating the adrenal gland functions, a control group and the cyantraniliprole treated group males were challenged with ACTH (12.5 μg) by tail vein injection on day 93. Blood and urine samples of these male rats were collected and analyzed for corticosterone levels. Body weights, food consumption, food efficiency, and clinical observations were evaluated weekly; acute clinical observations were conducted daily. Thyroid endpoints (hormone evaluations, anatomic pathology, organ weights, and hepatic biochemistry) were evaluated in females and adrenal endpoints (urine corticosterone, adrenal response to ACTH, organ weights, and anatomic pathology) were evaluated in males.

Under the conditions of this study, cyantraniliprole at 20000 ppm produced no effects on survival rates, food consumption, and clinical observation in either males or females. However, the 20000 ppm female rats showed lower body weight, body weight gain and food efficiency compared to controls.

In 20000 ppm females, increased liver and thyroid weights and minimal thyroid follicular cell hypertrophy were seen. These effects were associated with increased hepatic UDP-glucuronyltransferase (UDPGT) activity and alterations in thyroid hormone homeostasis including reduced serum T_4 concentration and increased TSH levels. These findings appeared to support the ideal that the thyroid effects seen in the 90-day study with cyantraniliprole might be a consequence of the increased in hepatic enzyme activity. The increased hepatic enzyme activity facilitated clearance of T_4, leading to lower T_4 levels, reduced negative feedback on hypothalamus and pituitary, and subsequent increased TSH stimulation of the thyroid gland. A reduction in hepatic microsomal 5′-deiodinase activity was also observed; however, the biological significance of this reduced activity was unclear, in the absence of any difference in T_3 or reverse T_3 (rT_3) levels.

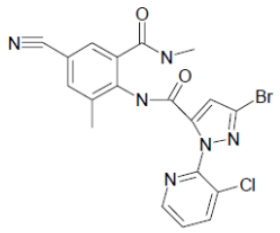
In 20000 ppm male rats, an increase in the incidence of microvesiculation (4/10) of the adrenal cortex with no evidence of cytotoxicity or degeneration was found. A minimal to mild increase in adrenal cytoplasm lipid vacuoles was observed by electron microscopy, but no effects on cellular organelles or evidence of cytotoxicity or degeneration were observed. In addition, microvesiculation was not associated with changes in adrenal cortical function, as basal urinary corticosterone and ACTH-induced serum corticosterone levels were comparable among all treatment groups.

The results generated in this study are valid and provide useful information in understanding the toxicology of cyantraniliprole.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Solid
CAS #	736994-63-1

Stability of test substance:	Analyses confirmed that the test material was stable in the feed, distributed uniformly, and present at the target concentrations.
2. Vehicle and/or positive control:	Untreated diet
3. Test animals	
Species:	Rat
Strain:	Crl:CD®(SD)
Age at start of dosing:	Approximately seven weeks old
Weight at start of dosing:	187.9–263.8 g for males; 158.1–190.6 g for females
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	4 or 11 days (two shipments)
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the food at concentrations specified by study design.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in solid-bottom caging with bedding and nestlet toys as enrichment.
4. Environmental conditions	
Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experimental start/completion

02-March-2009 to 20-May-2010

In-life initiated/completed

02-March-2009 to 03-June-2009

2. Animal assignment and treatment

Groups of 10 (males) or 15 (females) animals/sex/concentration were administered 0 (control) or 20000 ppm of cyantraniliprole in feed daily for 29 (females) or 93 (males) days (Table 1). Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means within a sex.

Table 1 Study Design

Males (93-day treatment duration)			Females (29-day treatment duration)		
No./ group	Conc. in diet (ppm)	Mean daily compound intakes [b] mg/kg bw	No./ group	Conc. in diet (ppm)	Mean daily compound intakes [b] mg/kg bw
10	0 [a]	0	15	0	0
10	20000 [a]	1230	15	20000	1903
10	0 [c]	0	--	—	—

a: For male rats in these 2 groups, on test day 93, ACTH was administered to each male rats by tail vein injection. Blood samples were collected for serum corticosterone measurements.
[b]: Data excerpted from pages 46 & 47 of the report.
[c] Control group 2 was included to evaluate non-ACTH-stimulated serum corticosterone levels

3. Diet preparation and analysis

The test substance was added to the rodent diet and thoroughly mixed for a period of time that by experience was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. All diets were prepared weekly or every other week and refrigerated until used. The homogeneity (beginning of study only) and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC at the beginning, in the middle, and at the end of the study. The test substance was at target concentrations ±13.0% and homogeneous (RSDs = 1 and 2%) throughout the feed. Stability of the test substance in the diet at this concentration was demonstrated in a concurrent study. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics: The statistical methods used are presented in Table 2.

Table 2. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, organ weight, mechanistic evaluations	Levene's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test

[a] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and morbidity and examined daily for clinical signs of toxicity.

2. Body weights

   All animals were weighed once per week and on the day of sacrifice.

3. Food consumption, food efficiency and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Biochemistry/ mechanistic parameters

   Thyroid evaluations

   Blood was collected, *via* the tail vein of female rats from each group, on test Day 14 and at sacrifice on test Day 29. Only test Day 29 samples were evaluated. Serum was prepared and stored frozen until analyzed for T_4, rT_3, and TSH concentrations. The liver, adrenal glands, and thyroid glands were collected from each female rat, weighed, and placed in appropriate fixative. Relative organ weights (% final body weight) were calculated. Thyroid glands from all female rats were processed to slides and examined microscopically by a veterinary pathologist. A portion of the liver from each of these animals was homogenized and hepatic microsomes prepared using differential centrifugation. Microsomal pellets were resuspended in homogenization buffer and stored frozen until analyzed for UDPGT and 5'-deiodinase activity. The protein content of the microsomes was determined before and after analysis by the Biorad method.

   Adrenal evaluations

   Urine was collected from male rats approximately 1 week before sacrifice (test day 86) to measure baseline (basal) urine corticosterone. On test day 93 (the day of necropsy for the male rats), ACTH (0.5 mL containing 12.5 μg ACTH) was administered to each male rat in groups 1 and 2 and saline was administered to each male rat in group 3, by tail vein injection. Approximately 60 minutes following intravenous injection of ACTH or saline, blood was collected from the orbital sinus of each male rat while it was under carbon dioxide anesthesia. Serum was prepared and stored between -60 and -80°C until analyzed for corticosterone concentrations. Following blood collection, the male rats were sacrificed and necropsied. Gross examinations were performed on all rats, and final body weights were recorded. The adrenal glands were collected from each rat, weighed, and placed in appropriate fixative. The right adrenal gland and half of the left adrenal gland of each female rat were placed in 10% neutral buffered formalin, routinely trimmed and processed to slides, and examined microscopically by a veterinary pathologist. A cortical section (*zona fasiculata*) of one adrenal gland from each of four male control and 20000 ppm rats was evaluated by transmission electron microscopy.

5. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anesthesia and exsanguination. Anatomic pathology was performed as described above.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs were observed in any male or female rats.

2. Mortality

All animals survived to scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Cyantraniliprole produced no statistically significant changes in body weights of males which were treated for 93 day and females (28-day treatment). There was a reduction in body weight gain in females which received cyantraniliprole at 20000 ppm for 28 days (↓21%).

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

No treatment-related changes in food consumption were observed in males or females. Mean overall food efficiency in 20000 ppm males and females was 94% (not statistically significant) and 78% (statistically significant) of control, respectively.

D. MECHANISTIC PARAMETERS

1. Thyroid evaluation - females

Statistically significant increases in serum TSH levels occurred at 20000 ppm (↑67%) (Table 3). Statistically significant decreases in T4 levels (↓30% were observed. Serum levels of T3 and rT3 were unaffected. Statistically significant higher hepatic microsomal UDPGT activity (↑77%) was observed in female rates exposed to 20000 ppm. Statistically significant decreases in hepatic microsomal 5'-deiodinase activity (↓23%) were observed in female rats exposed to 20000 ppm.

Table 3. Thyroid and biochemical parameters for female rats.

Parameter	0 ppm	20000 ppm

T4 (µg/dL)	3.16 ±0.63	2.22 ± 0.47[a](↓30%)
T3 (ng/dL)	75.00± 8.30	73.2± 9.9
TSH (ng/dL)	1.68± 0.39	2.81± 0.64[b](↑67)
rT3 (ng/mL)	0.114± 0.024	0.115 ± 0.019
5'deiodinase (nmole/hr/mg protein)	6.80 ± 0.66	5.25± 0.85[a](↓23%)
UDP-GT (nmole/min/mg protein)	11.6± 3.5	20.5± 5.5[a](↑77%)

Data excerpted from pages
[a] Statistically significant, $p<0.05$.

2. Adrenal evaluation - males

Urine levels of corticosterone (absolute or per mL urine) and corticosterone/creatinine ratio were not statistically significantly different among the test groups (Table 4). As expected, serum corticosterone concentrations in rats challenged with ACTH were much higher than those in rats not challenged with ACTH. There was no statistically significant difference between ACTH challenged rats exposed to test substance in the diet compared to those fed control diet. Therefore, the test substance had no effect on the ability of the adrenal gland to respond to ACTH stimulation with increased serum corticosterone production (Table 5).

Table 4. Summary of urine corticosterone levels in male rats challenged with ACTH (12.5 µg)

	0 ppm[a]	**0 ppm + µg ACTH 12.5 µg**	**cyantraniliprole 20000 ppm + ACTH 12.5 µg**
Corticosterone (ng/mL)	64.7±51	84.2±74	87.2±56
Corticosterone (ng)	556±184	448±177	649±291
Corticosterone: creatinine ratio (%)	40.7±10	44.2±27	56.8±28

Data excerpted from pages 51 of the report
[a] Control group not receiving ACTH.

Table 5. Summary of serum corticosterone levels in male rats challenged with ACTH (12.5 µg)

	0 ppm[a]	**0 ppm + ACTH 12.5 µg**	**cyantraniliprole 20000 ppm + ACTH 12.5 µg**
Corticosterone (ng/mL)	87±43	410±54	473±129

Data excerpted from pages 51 of the report.
[a] Control group not receiving ACTH.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

Females

Mean absolute liver and relative liver weights were increased 22 and 30%, respectively, in the 20000 ppm group as compared to the control values. Both increases were statistically significant. Mean absolute and relative thyroid weights were increased 21 and 17%, respectively, in 20000 ppm group as compared to the control values. Although the increases in mean absolute and relative thyroid weight in 20000 ppm female rats were not statistically significant, these were considered test substance related because increased thyroid weight correlated microscopically with thyroid follicular cell hypertrophy. Mean relative adrenal weights were increased 17% in the 20000 ppm group as compared to the control values.

Table 6. Organ weights for female rats.

Parameter	0 ppm	20000 ppm
Liver absolute weight (g)	10.0±	**12.2±0.9[b](↑22%)**
Relative liver weight (%)	4.1	**5.3[a]**
Thyroid absolute weight (g)	0.014±0.004	**0.017±0.004(↑21%)**
Relative thyroid weight (%)	0.006	**0.007**
Adrenal absolute weight (g)	0.056±0.008	0.062±0.006(↑11%)
Relative adrenal weight (%)	0.023	0.027[b]

[a] Statistically significant (Dunnett/Tamhane-Dunnett prametric comparison to control).
[b] Statistically significant (Dunn's non-parameteric comparison to control).
Note: Bolded values were interpreted to be test substance-related effects.
Data excerpted from pages 53 of the report.

Males

There were no test substance-related effects on absolute or relative adrenal gland weight.

2. Gross and microscopic pathology

Females

There were no test substance-related gross observations. Microscopically, test substance-related microscopic thyroid follicular cell hypertrophy was observed in female rats (Table 7).

Table 7. 28-day treatment: Incidences of thyroid follicular cell hypertrophy in female rats

Cyantraniliprole (ppm):	0	20000

Thyroid follicular cell hypertrophy	0/15	**5/15**

Note: Bolded values were interpreted to be test substance-related effects. Data excerpted from page 55 of the study report.

Males

There were no test substance-related gross observations. Microscopically, there was a small increase in the incidence of adrenal cortical microvesiculation in the 20000 ppm females rats as compared to both control group rats (Table 8).

Table 8. 90-day treatment: Incidences of adrenal gland microvesiculation in male rats

Cyantraniliprole (ppm):	**0[a]**	**0[b]**	**20000**
Adrenal gland microvesiculation (zona fasciculata)	0/10	0/10	**4/10**

[a] Control group receiving ACTH. Data excerpted from page 55 of the study report.

[b] Control group not receiving ACTH.

Note: Bolded values were interpreted to be test substance-related effects.

3. Electron microscopic pathology

A minimal to mild increase in adrenal cytoplasm lipid vacuoles was observed by electron microscopy in cyantraniliprole-treated male rats in relationship to control rats, but no effects on cellular organelles or evidence of cytotoxicity or degeneration were observed.

III. CONCLUSION

The data of this study showed that cyantraniliprole at 20000 ppm induced an increase in hepatic UDP-glucuronyl transferase activity, a decrease in T_4 levels, and an increase in TSH level in test females. These findings suggest that the increase in liver enzyme activity resulting in increased clearance of T_4. Lower T_4 level reduces negative feedback on the hypothalamus and pituitary resulting in an increased TSH release. TSH stimulates the thyroid gland leading to thyroid follicular cell hypertrophy. A reduction in hepatic microsomal 5'-deiodinase activity was also observed; however, the biological significance of this reduced activity is unclear, in the absence of any difference in T_3 or rT_3.

The responses of male rats to 20000 ppm dietary cyantraniliprole demonstrated that the test substance did not affect adrenal cortical cell structure or function at an exposure level that produced increased adrenal cortex microvesiculation.

Revised by US EPA (Global Primary Reviewer: Whang Phang, PhD)

IIA 5.8/04

Report: Clarke, J.J. (2009); IN-JSE76: *In vitro* mammalian cell gene mutation test (CHO/HGPRT Assay). BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.: AC22CC.782.BTL. DuPont Report No.: DuPont-24714. Study Completion Date: February 04, 2009. MRID 48119974. Unpublished.

Guidelines: OPPTS 870-5300
ECC 2000/32/EC, Annex 4E No. L136
OECD 476 (1998)
JMAFF 59-Nousan-4200 (1985)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In a CHO/HGPRT mutation assay (MRID 48119974), IN-JSE76 (93.8% purity) was tested in the presence and absence of an exogenous metabolic activation system (Aroclor-induced rat liver S9). Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K_1 cells were exposed for 5 hours at 37 ± 1°C to the test substance at concentrations of 0, 100, 150, 500, 1000, and 1500 μg/mL. The highest concentration level was set based on insolubility of the test substance at concentrations ≥1500 μg/mL. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-thioguanine (2-amino-6-mercaptopurine)-resistant phenotype. The test substance was dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration of 250 mg/mL. Ethyl methanesulfonate (EMS) and Benzo(a)pyrene (B(a)P) were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of <50% of the solvent control. The assay was considered positive when a dose-dependent increase in mutation frequencies occurred with at least 2 consecutive doses having mutation frequencies of greater than 40 mutants per 10^6 clonable cells.

In the mutagenesis assay, visible precipitate was observed at concentrations ≥1500 μg/mL at the end of treatment. No toxicity was observed at any concentration, and no positive responses were observed..

IN-JSE76 was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for an *in-vitro* mammalian cell gene mutation assay (OPPTS 870-5300; OECD 476).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JSE76 technical metabolite
   Lot/Batch #: JSE76-005
   Purity: 93.8%
   Description: Off-white to light brown powder
   CAS #: Not available
   Stability of test compound: Results from analysis of the dosing solutions indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)
   Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of .2 μL/mL
   Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4 μg/mL
3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2178 and 2313
   Source: Moltox, Inc., Boone, NC
   Protein content: 41.7 mg/mL
   Source: None
   Characterisation: The metabolic activation ability of the S9 was characterised by its ability to metabolize at least 2 pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition
   NADP: 4 mM
   Glucose-6-phosphate: 5 mM
   KCl: 30 mM
   $MgCl_{2:}$ 10 mM
   Sodium phosphate buffer (pH 8.0): 50 mM
   Calcium chloride: 10 mM
   S9 homogenate: 10% (v/v)

4. Test cells

   Chinese Hamster Ovary cells (CHO-K1) were properly maintained, periodically checked for mycoplasma contamination, and periodically “cleansed” against high spontaneous background. Cells used in the mutation assay were within four subpassages from cleansing in order to assure karyotypic stability.

5. Culture medium

F12FBS5-Hx (Ham's F12 medium without hypoxanthine supplemented with 5% dialyzed foetal bovine serum (FBS), 100 units penicillin/mL, 100 μg streptomycin/mL and 2 mM L-glutamine/mL.

6. Locus examined

Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) – the selection agent used was 10 μM 6-thioguanine (6TG, 2-amino-6-mercaptopurine).

7. Test compound concentrations used

Preliminary cytotoxicity assay:	Concentrations of 0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 2500 μg IN-JSE76/mL were evaluated in the presence and absence of S9 activation.
Mutagenesis assay:	Concentrations of 100, 150, 500, 1000, and 1500 μg IN-JSE76/mL were evaluated in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion:

November 19, 2008 to December 29, 2008

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and nine concentrations of test substance ranging from 0.5 to 2500 μg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenesis assay

CHO cells were exposed for 5 hours to the vehicle alone, appropriate positive controls and seven concentrations of test substance in duplicate in both the absence and presence of S9-activation. Duplicate flasks of cells were exposed to six concentrations of the test substance for 5 hours at 37 ± 1°C. After 5 hours, the cells were washed with Ca^{++} and Mg^{++} – free Hanks' balanced salt solution (HBSS) and cultured for an additional 18 to 24 hours. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

Cytotoxicity: The replicates from each treatment condition were detached using trypsin and subcultured in triplicate at a density of 100 cells/60 mm dish. After 7 to 10 days incubation, the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted and cloning efficiency determined.

Phenotypic expression/selection: The replicates from each treatment condition were detached using trypsin and subcultured in duplicate at a density no greater than 10^6 cells/100 mm dish. Subculturing at 2 to 4 day intervals was employed for the 7 to 9 day

expression period. For selection of the TG-resistant phenotype, the replicates from each treatment condition were trypsinised and replated, in quintuplicate, at a density of 2×10^5 cells/100 mm dish in medium containing 10 μM 6-thioguanine (TG, 2-amino-6-mercaptopurine). For cloning efficiency determination at the time of selection, 100 cells/60-mm dish were plated in triplicate. After 7 to 10 days of incubation, the colonies were fixed, stained and counted for both cloning efficiency and mutant selection.

4. Statistics

The data did not warrant statistical analysis.

5. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies with at least two consecutive doses showing mutant frequencies of >40 mutants per 10^6 clonable cells. If a single point above 40 mutants per 10^6 clonable cells was observed at the highest dose, the assay was considered suspect. If no culture exhibited a mutant frequency of >40 mutants per 10^6 clonable cells, the test substance was considered negative.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was workable in DMSO at a maximum concentration of 250 mg/mL with sonication and soluble at 200 μg/mL. Visible precipitate was present at 2500 μg/mL in the treatment medium at the end of treatment. Cloning efficiency relative to the solvent controls (relative cloning efficiency) was 102% at 2500 μg/mL without activation and 83% at 2500 μg/mL with S9 activation. Based on these findings and the precipitation profile of the test substance, the doses chosen for the mutagenesis assay ranged from 100 to 2500 μg/mL for both the non-activated and S9-activated cultures.

B. MUTAGENESIS ASSAY

Visible precipitate was observed at concentrations ≥1500 μg/mL. (Cultures treated with 2000 and 2500 μg/mL were not cloned due to precipitation.) The results are summarized in Table 1 and the details are shown in Tables 2 and 3. Relative cloning efficiency was 93% and 105% at the highest dose cloned for mutant selection in the non-activated and S9-activated systems, respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 10^6 clonable cells.

Table 1. *In vitro* mammalian gene mutation: Summary of mutagenicity findings

Compound	Conc. µg/mL	Mutation frequency without activation[a]	Mutation frequency with activation[a]
IN-JSE76	0	7.4	10.0
	100	8.2	0
	150	1.7	19.8
	500	0	0.9
	1000	1.9	0
	1500[b]	10.7	6.7
EMS (0. µL/mL)		272.0	ne
B(a)P (4 µg/mL)		ne	80.8

[a] Mutants per 1×10^6 surviving cells defined as: total mutant colonies/(number of selection dishes × cloning efficiency × 2×10^5 cells) × 10^6

[b] Precipitating concentration

EMS = ethyl methanesulfonate (0.2 µL/mL)

B(a)P = benzo(a)pyrene (4 µg/mL)

ne = Not evaluated

Data excerpted from page 20 of the study report.

III. CONCLUSION

IN-JSE76 was tested up to 1500 µg/mL, which resulted in visible precipitation, but failed to induce a mutagenic response in CHO cells in either the absence or the presence of S9 activation. The positive controls produced the expected mutagenic responses. It was concluded that IN-JSE76 was negative in the non-activated and S9-activiated test system in the CHO/HGPRT mutation assay.

Table 2. Non-activated (-S9) Study using IN-JSE76

(Table excerpted from page 19 of the study report)

Cloning Efficiency Plates						Cloning Efficiency	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells
Treatment (µg/mL)	Subset	Plate Counts 1	2	3	Average Colonies		Plate Counts 1	2	3	4	5	Average Colonies	
Solvent	A	50	53	75	60.8	0.61	0	0	0	0	0	0.9	**7.4**
	B	73	53	*			0	6	0	3	0		
EMS (0.2µL/mL)	A	54	62	51	52.5	0.53	34	27	28	28	*	28.6	**272.0**
	B	37	46	65			28	33	23	31	25		
100	A	61	60	32	60.7	0.61	0	0	2	0	0	1.0	**8.2**
	B	64	71	76			1	1	2	3	1		
150	A	63	62	56	60.3	0.60	0	2	0	0	0	0.2	**1.7**
	B	56	67	58			0	0	0	0	0		
500	A	*	83	*	72.0	0.72	0	0	0	0	0	0	**0**
	B	66	75	64			0	0	0	0	*		
1000	A	95	103	111	78.3	0.78	0	0	0	0	0	0.3	**1.9**
	B	58	63	40			1	1	1	0	0		
1500 P	A	68	76	30	60.8	0.61	0	1	5	2	1	1.3	**10.7**
	B	69	59	63			0	0	0	1	3		

A and B are duplicate cultures
P - Precipitating concentration
* - Culture lost to contamination

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

Table 3. Activated (+S9) Study using IN-JSE76

(Table excerpted from page 20 of the study report)

Cloning Efficiency Plates						Cloning Efficiency	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells
Treatment (µg/mL)	Subset	Plate Counts 1	2	3	Average Colonies		Plate Counts 1	2	3	4	5	Average Colonies	
Solvent	A	*	46	*	55.3	0.55	0	1	0	0	0	1.1	**10.0**
	B	62	57	56			1	1	1	4	3		
B(a)P (4µg/mL)	A	*	52	*	44.0	0.44	4	7	7	6	7	7.1	**80.8**
	B	45	40	39			5	8	9	11	*		
100	A	*	51	*	56.3	0.56	0	0	0	0	0	0	**0**
	B	50	68	*			0	0	0	0	0		
150	A	46	66	*	59.0	0.59	14	2	2	*	3	2.3	**19.8**
	B	48	76	*			0	0	0	0	0		
500	A	51	41	*	58.0	0.58	0	0	1	0	0	0.1	**0.9**
	B	83	57	*			0	0	0	0	0		
1000	A	*	52	*	47.0	0.47	0	0	0	0	0	0	**0**
	B	42	*	*			0	0	0	0	0		
1500 P	A	58	66	55	58.0	0.58	0	0	2	1	0	0.8	**6.7**
	B	64	47	*			1	0	1	2	*		

A and B are duplicate cultures
P - Precipitating concentration
* - Culture lost to contamination

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

Revised by US EPA (Global Primary Reviewer: Whang Phang, PhD)

IIA 5.8/07

Report: Gudi, R., Rao, M. (2010); IN-JSE76: *In vitro* mammalian chromosome aberration test. BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.: AC22CC.341.BTL. DuPont Report No.: DuPont-24715, Revision No. 2. Study Completion Date: January 30, 2009. Revised Report Completion Date: July 07, 2009. Second Revised Report Completion Date: March 12, 2010. MRID 48119975. Unpublished.

Guidelines: OPPTS 870-5375
EU 2000/32/EC, L136, Annex 4A-B10
OECD 473 (1998)
JMAFF (November 24, 2000 and later revisions)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In an *in vitro* mammalian chromosome aberration assay (MRID 48119975), IN-JSE76 (93.8% purity) was tested using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). The test substance was soluble in dimethyl sulfoxide (DMSO) at a maximum concentration of approximately 200 mg/mL in the solubility test. HPBL were treated for 4 hours with S9-activated test system, and 4 and 20 hours with non-activated test system. Visible precipitate was observed in treatment medium at 2500 μg/mL, and concentration levels ≤750 μg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. In the preliminary toxicity test, the cells were exposed to IN-JSE76 at concentrations ranging from 0.26 to 2500 μg/mL as well as the vehicle control. Substantial toxicity (i.e., ≥ 50% reduction in mitotic index relative to the solvent control) was not observed at any dose level in the non-activated 4-hour exposure group. However, Substantial toxicity was observed at dose levels ≥750 μg/mL in the S9-activated 4-hour exposure group and at 2500 μg/mL in the non-activated 20-hour exposure group. Based on these observations, the concentrations chosen for the chromosome aberration assay were the following: for the non- activated 4-hour exposure group: 0, 313, 625,1250, and 2500 μg/mL; for the 20-hour exposure non-activated group: 156, 313, 625, 1000, 1500, and 2000 μg/mL; for 4-hour exposure S9-activated group: 156, 313, 625, 1250, and 2500 μg/mL. After exposure to Colcemid®, metaphase cells were harvested approximately 20 hours following the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition) then structural and numerical chromosome aberrations.

In the chromosome aberration assay, visible precipitate was observed in treatment medium at dose levels ≥1000 μg/mL, and dose levels ≤625 μg/mL were soluble in treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, in the non-activated and S9-activated 4-hour exposure groups, visible precipitate was observed in treatment medium at 2500 μg/mL, and dose levels ≤1250 μg/mL were soluble in treatment medium. In the

non-activated 20-hour exposure group, visible precipitate was observed in treatment medium at 2000 μg/mL and dose levels ≤1500 μg/mL were soluble in treatment medium.

In the S9-activated 4-hour and the non-activated 20-hour exposure groups, selection of doses for microscopic analysis was based on mitotic inhibition (the lowest dose with at least 50% reduction in mitotic index, relative to the solvent control and two lower doses). In the non activated 4-hour exposure group, selection of doses for microscopic analysis was based on precipitation of the test substance in the treatment medium (the dose with the least precipitation and two lower doses). The percentage of cells with structural or numerical aberrations in the test substance treated groups did not show statistically significant increase relative to the solvent control at any dose level.

Based on the results of this study, IN-JSE76 was concluded to be negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes with and without exogenous activation system.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for an *in vitro* mammalian cytogenetics (chromosome aberration) assay.

MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-JSE76 technical metabolite
	Lot/Batch #:	JSE76-005
	Purity:	93.8%
	Description:	Off white to light brown powder
	CAS #	Not available
	Stability of test compound:	Results from analysis of the dosing solutions indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
2.	Control materials	
	Solvent control:	Dimethyl sulfoxide (DMSO)
	Positive, non-activation:	Mitomycin C (MMC) in water at 30 and 60 μg/mL
	Positive, activation:	Cyclophosphamide (CP) in water at 20 and 40μg/mL
3.	Activation:	Rat liver S9 from Male Sprague-Dawley rats (10% Aroclor 1254-induced)
	Lot number:	2278 and 2178
	Source:	Moltox, Inc., Boone, NC
	Protein content:	Not given
	Source:	Not applicable

Characterisation:	The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.
S9 mix composition	
Sodium phosphate buffer (pH 7.4):	Not applicable
Glucose-6-phosphate:	1 mM
NADP:	1 mM
KCl:	6 mM
$MgCl_{2:}$	2 mM
S9:	20 µL per mL medium

4. Test cells

   Human lymphocytes obtained from human venous blood from normal, healthy donors.

5. Culture medium

   RPMI 1640, supplemented with 1% phytohaemaglutinin (PHA), 100 units penicillin/mL and 100 µg streptomycin/mL, and 2 mM L-glutamine.

6. Test compound concentrations evaluated

Non-activated and S9-activated 4-hour exposure groups:	Concentrations of 625, 1250, and 2500 µg IN-JSE76/mL in duplicate in the presence and absence of S9 activation.
Non-activated 20-hour exposure group:	Concentrations of 313, 625, and 1000 µg IN-JSE76/mL in duplicate in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   November 13, 2008 to November 29, 2008

2. Preliminary cytotoxicity assay

   The toxicity test was performed for the purpose of selecting concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, number of dividing cells/500 cells counted). The cells were exposed to solvent alone and to concentrations of the test substance ranging from 0.25 to 2500 µg/mL for 4 hours in both the presence and absence of S9 activation and for 20 hours continuously in the absence of S9 activation.

3. Cytogenetic Assay

   Cell treatment:

   Based on the results of the preliminary cytotoxicity test, cells were exposed to test

compound, solvent or positive control for 4 h or 20 h (non-activated) or 4 h (activated) as shown below:

Treatment condition	Treatment time (h)	IN-JSE76 Concentrations (μg/mL)	Positive controls	
			Mitomycin C (MMC) (μg/mL)	Cyclophosphamide (CP) (μg/mL)
Non-activated:	4 hr	313, 625,1250, & 2500	0.6	
	20 hr	156, 313, 625, 1000, 1500, & 2000	0.3	
S-9 activated:	4 hr	156, 313, 625, 1250, & 2500		20

Spindle inhibition

Two hours prior to the scheduled cell harvest, Colcemid® was added to the cell cultures at a final concentration of 0.1 μg/mL.

Cell harvest

Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation at 1200 rpm for about 5 minutes. The cell pellet was resuspended in 5 mL of 0.075 M KCl and incubated at 37 ± 1°C for 20 minutes. At the end of the KCl treatment and immediately prior to centrifuging, the cells were gently mixed and approximately 0.5 mL of fixative (methanol:glacial acetic acid, 3:1 v/v) was added to each tube. The cells were collected by centrifugation, the supernatant aspirated, and the cells were fixed with two washes with approximately 3 to 5 mL of fixative and stored in fixative overnight or longer at approximately 2 to 8°C.

Slide preparation

Fixed cells were centrifuged at approximately 1200 rpm for 5 minutes, the supernatant was aspirated, and the cells were suspended in 1 mL cold fresh fixative. The cells were collected by centrifugation and the supernatant aspirated, leaving 0.1 to 0.3 mL fixative above the cell pellet. One to two drops of the cell suspension were dropped onto a glass slide and allowed to air dry at room temperature. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Selection of Dose Levels for Analysis

In the S9-activated 4-hour and the non-activated 20-hour exposure groups, selection of doses for microscopic analysis was based on mitotic inhibition (the lowest dose with at least 50% reduction in mitotic index, relative to the solvent control and two lower doses). In the non-activated 4-hour exposure group, selection of doses for microscopic analysis was based on precipitation of the test substance in the treatment medium (the dose with the least precipitation and two lower doses).

Evaluation of metaphase cells

Metaphase cells with 46 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverised chromosome(s), pulverised cells and severely damaged cells (≥10 aberrations) also were recorded. Chromatid gaps and isochromatid gaps were recorded but not included in the analysis. The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted. The percentage of polyploid and endoreduplicated cells was evaluated per 100 cells.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's Exact test. Fisher's Exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's Exact test at any test substance dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

5. Evaluation criteria

The test substance was considered to induce a positive response when the percentages of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historical solvent controls may be judged as not biologically significant. Test substances not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

IN-JSE76 was present at acceptable concentrations in the dosing solutions (within 104% of nominal concentrations). IN-JSE76 was shown to be stable in the dosing solutions under the conditions of the study. IN-JSE76 was not found in the 0 mg/mL samples. The positive and solvent controls fulfilled the requirements for a valid test.

B. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of approximately 200 mg/mL in the solubility test. Visible precipitate was observed in treatment medium at 2500 μg/mL, and dose levels ≤750 μg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was not observed at any dose level in the non-activated 4-hour exposure group (Table 1). However, marked toxicity was observed at dose levels ≥750 μg/mL in the S9-activated 4-hour exposure group and at 2500 μg/mL in the non-activated 20-hour exposure group.

C. CHROMOSOME ABERRATION ASSAY (Tables 2 and 3)

Table 2 summarizes the results of the assays. In the chromosome aberration assay, visible precipitate was observed in treatment medium at ≥1000 μg/mL and dose levels ≤625 μg/mL were soluble in treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, in the non-activated and S9-activated 4-hour exposure groups, visible precipitate was observed in treatment medium at 2500 μg/mL and dose levels ≤1250 μg/mL were soluble in treatment medium. In the non-activated 20-hour exposure group, visible precipitate was observed in treatment medium at 2000 μg/mL and dose levels ≤1500 μg/mL were soluble in treatment medium.

Non-activated 4 h exposure group:

At the highest test concentration evaluated microscopically for chromosome aberrations, 2500 μg/mL, mitotic inhibition was approximately 19%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated group was not increased relative to the solvent control ($p > 0.05$, Fisher's exact test).

Activated 4 h exposure group:

At the highest test concentration evaluated microscopically for chromosome aberrations, 2500 μg/mL, mitotic inhibition was 55%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated group was not increased relative to the solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

Non-activated 20 h exposure group:

At the highest test concentration evaluated microscopically for chromosome aberrations, 1000 μg/mL, mitotic inhibition was 50%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated groups was not increased relative to the solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

III. CONCLUSIONS

IN-JSE76 was negative for structural and numerical chromosome aberrations in the non-activated and S9-activated test systems in the *in vitro* test mammalian chromosome aberration assay using human peripheral blood lymphocytes.

Table 1 Summary result of the toxicity test
(Data excerpted from pages 25-27 of the study report)

4-HOUR TREATMENT

Treatment (-S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	12.2	
IN-JSE76		
0.25	12.0	-2
0.75	12.4	2
2.5	12.0	-2
7.5	11.8	-3
25	11.0	-10
75	10.0	-18
250	11.0	-10
750	10.8	-11
2500	9.8	-20

4-HOUR TREATMENT

Treatment (+S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	9.0	
IN-JSE76		
0.25	8.8	-2
0.75	8.8	-2
2.5	7.6	-16
7.5	8.0	-11
25	6.0	-33
75	7.2	-20
250	6.8	-24
750	4.4	-51
2500	2.8	-69

20-HOUR TREATMENT

Treatment (-S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	12.6	
IN-JSE76		
0.25	11.6	-8
0.75	11.0	-13
2.5	11.0	-13
7.5	11.8	-6
25	10.8	-14
75	10.8	-14
250	10.6	-16
750	11.0	-13
2500	2.2	-83

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Mitotic Index = (Cells in mitosis/500 cells scored) x 100.
Percent change = (Treatment mitotic index - control mitotic index)/control mitotic index, expressed as a percentage.

Table 2. Detailed summary of the chromosome aberration test
(Table excerpted from page 31 of the study)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	12.4	200	200	0.000	±0.000	0.0	0.0
IN-JSE76									
625	-S9	4	12.0	200	200	0.000	±0.000	0.0	0.0
1250	-S9	4	10.6	200	200	0.000	±0.000	0.0	0.0
2500	-S9	4	10.0	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	6.1	200	100	0.170	±0.403	0.0	16.0**
DMSO	+S9	4	11.0	200	200	0.000	±0.000	0.0	0.0
IN-JSE76									
625	+S9	4	8.7	200	200	0.000	±0.000	0.0	0.0
1250	+S9	4	6.8	200	200	0.000	±0.000	0.0	0.0
2500	+S9	4	4.9	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	2.4	200	100	0.290	±0.756	0.0	19.0**
DMSO	-S9	20	12.3	200	200	0.000	±0.000	0.0	0.0
IN-JSE76									
313	-S9	20	10.9	200	200	0.000	±0.000	0.0	0.0
625	-S9	20	11.1	200	200	0.000	±0.000	0.0	0.0
1000	-S9	20	6.2	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	6.4	200	100	0.260	±0.597	0.0	20.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using the Fisher's Exact test.

Revised by US EPA (Global Primary Reviewer: Whang Phang, PhD)

IIA 5.8/13

Report: Wagner, V.O., VanDyke, M.R. (2009a); IN-JSE76: Bacterial reverse mutation assay. BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.: AC22CC.503.BTL. DuPont Report No.: DuPont-24716. Study Completion Date: February 25, 2009. MRID 48119976. Unpublished.

Guidelines: OPPTS 870-5100
ECC 2000/32/EC (2000)
OECD 471 (1998)
JMAFF 12-Nousan-8147 (2000 or later revisions)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In a bacterial reverse muatation study (MRID 48119976.) IN-JSE76,(93.8% purity) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA 98, TA100, TA1535, and TA1537 and in *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) (MRID 48119976). The first phase, initial toxicity-mutation assay (Trial I) of the study was conducted to determine the dose range and toxicity of IN-JSE76 under the assay conditions and to provide a preliminary mutagenicity evaluation. The second phase (Trial II) was to evaluate and to confirm the mutagenic potential of the test substance. Based on the solubility of the test substance and compatibility with the target cells, dimethyl sulfoxide (DMSO) was selected as the solvent

In the initial toxicity-mutation assay, the dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg per plate. Neither precipitate nor appreciable toxicity was observed, and no mutagenic response was observed. In the confirmatory mutagenicity assay, the dose levels tested were 50, 150, 500, 1500, and 5000 μg per plate, and no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was found.

Under the conditions of this study, IN-JSE76 was negative for bacterial mutagenic activity in non-activated and S9-activated test systems. This study is fully reliable (acceptable/guideline) and satisfies the requirements for a bacterial mutation assay (OPPTS 870-5100; OECD 471).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JSE76 technical metabolite
   Lot/Batch #: JSE76-005
   Purity: 93.8%
   Description: Solid
   CAS # Not available
   Stability of test compound: Results from analysis of the dosing solutions from acceptable trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)

Positive control, non activation:

Positive control	Dose	Strain(s)
2-nitrofluorene	1 µg/plate	TA98
sodium azide	1 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-aminoanthracene	1[a] µg/plate	TA98, TA100, TA1535, and TA1537
	10 µg/plate	WP2 *uvr*A

[a] 2.0 µg per plate was used for tester strain TA100 in Experiment B4.

3. Activation:	Rat liver S9 from male Sprague-Dawley rats (10% Aroclor 1254-induced)
Lot number:	2359
Source:	Moltox, Inc., Boone, NC
Protein content:	Not given
Source:	Not applicable
Characterisation:	The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.
S9 mix composition	
Sodium phosphate buffer (pH 7.4):	100 mM
Glucose-6-phosphate:	5 mM
NADP:	4 mM
KCl:	33 mM
$MgCl_{2:}$	8 mM
S9:	10% (v/v)

4. Test organisms

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

Initial toxicity-	Concentrations of 1.5, 5.0, 15, 50, 150, 500, 1500, and
Mutation assay (Experiment B1):	5000 μg IN-JSE76/plate were evaluated in duplicate in the presence and absence of S9 activation.
Confirmatory mutagenicity assay (Experiment B4):	Concentrations of 50, 150, 500, 1500, and 5000 μg IN-JSE76/plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

December 02, 2008 to January 19, 2009

2. Plate incorporation assay

This study consisted of 2 independent phases that assessed test substance mutagenicity. In the first phase, 2 replicates were plated for each tester strain in the presence and absence of the exogenous metabolic activation system at each test substance

concentration. The second phase was conducted on triplicate plates. Positive and vehicle controls were included for each strain with and without activation. Treatments with the exogenous metabolic activation system were conducted by adding 50 µL of appropriate positive control or test substance dilution, 0.5 mL of metabolic activation system, and 100 µL of an overnight culture containing greater than 0.3×10^9 cells/mL of bacteria to approximately 2 mL of top agar (0.8% [w/v] agar and 0.5% NaCl [w/v]) supplemented with L-histidine, D-biotin, and L-tryptophan solution to a final concentration of 50 µM each. These components were briefly mixed and poured onto a minimal glucose agar plate. Treatments in the absence of the metabolic activation system were the same as those in the presence of the exogenous metabolic activation system with the exception that 0.5 mL of sterile phosphate buffer was used as a replacement for the volume of the exogenous metabolic activation system. After pouring onto the surface of minimal glucose agar plates, the top agar was allowed time to solidify and the individually labelled plates were inverted and incubated at approximately 37°C for about 48 to 72 hours. Plates were refrigerated at approximately 2 to 8°C prior to evaluation and counting of revertant colonies.

Bacterial background lawns were evaluated for evidence of test substance toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity.

3. Statistics

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated, no further statistical analyses were conducted.

4. Evaluation criteria

For the test substance to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test substance. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100, and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

IN-JSE76 was present at acceptable concentrations in the dosing solutions (within 86.5% of nominal concentrations) in the confirmatory trial deemed acceptable. IN-JSE76 was shown to be stable in the dosing solutions under the conditions of the study. IN-JSE76 was not found in the 0 mg/mL samples.

B. MUTATION ASSAYS

Based on the solubility of the test substance and compatibility with the target cells, dimethyl sulfoxide (DMSO) was selected as the solvent. After sonicating the mixture of IN-JSE76 and DMSO for 40 minutes at 27°C, the mixture formed a suspension at approximately 250 mg/mL and a soluble and clear solution in DMSO at approximately 200 mg/mL. No precipitate or appreciable toxicity was observed at any test concentration, and no positive mutagenic responses were observed in the initial assay (B1). In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in the absence of S9 activation or with tester strains TA98, TA100, TA1535, and WP2 *uvr*A in the presence of S9 activation. The dose levels tested were 50, 150, 500, 1500, and 5000 μg per plate. Neither precipitate nor appreciable toxicity was observed (B2). Due to an unacceptable positive control value, tester strain TA1537 in the presence of S9 activation was not evaluated but was retested using the same dose levels (B3). Neither precipitate nor appreciable toxicity was observed. The concentration analysis from Experiment B2 indicates that the actual concentrations of the analyzed dose levels (1.0, 10, and 100 mg/mL) were lower than expected (approximately 29, 50, and 86% of their respective targets). Due to the abnormal differences from nominal concentrations of the dosing formulations, the confirmatory mutagenicity assay was retested (B4) and the samples from Experiment B3 were not analyzed. Data generated in Experiments B2 and B3 were not reported and were not used in the evaluation of this test substance. The results of the confirmatory mutagenicity assay were data that were generated in Experiment B4. The dose levels tested were 50, 150, 500, 1500, and 5000 μg/plate. Neither precipitate nor appreciable toxicity was observed. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

Table 1. Summary of average revertants/plate without activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Phase I[a]	Phase II[b]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
IN-JSE76	0	13	16	146	146	11	9	9	6	19	31
	1.5	11	ne	126	ne	10	ne	9	ne	21	ne
	5.0	10		121		13		12		19	
	15	13		115		11		15		19	
	50	9	23	110	150	10	15	9	5	14	29
	150	12	14	121	140	12	13	11	5	25	28
	500	13	20	147	134	14	14	9	6	19	32
	1500	13	15	127	152	10	17	7	6	21	24
	5000	16	17	128	159	11	15	11	4	28	27
2NF	—	225	170	ne							
NAAZ	—	ne		648	714	424	545				
9AA	—	ne						1403	1203	ne	
MMS	—	ne								237	372

[a] Average of 2 replicates per trial. Phase I : initial toxicity mutation assay (Experiment B1).
[b] Average of 3 replicates per trial. Phase II confirmatory mutagenicity assay (Experiment B4)
2NF = 2-nitrofluorene; NAAZ = sodium azide; 9AA = 9-aminoacridine; MMS = methyl methanesulfonate
ne = not tested in this strain of bacteria.
Data excerpted from pages 41 & 42 of the study report.

Table 2. Summary of average revertants/plate with activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Phase I[a]	Phase II[b]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
IN-JSE76	0	17	27	139	172	14	14	10	8	19	38
	1.5	20		131		13		11		21	
	5.0	12	ne	143	ne	12	ne	11	ne	25	ne
	15	20		144		10		5		17	
	50	19	32	155	161	16	12	6	9	18	33
	150	10	25	138	171	13	11	5	6	24	33
	500	18	27	148	151	14	13	7	6	21	32
	1500	17	27	164	161	16	14	6	7	15	33
	5000	16	26	135	140	9	14	8	5	15	31
2AA	----	699	451	665	1400	134	111	109	74	428	427

[a] Average of 2 replicates per trial. Phase I : initial toxicity mutation assay (Experiment B1).
[b] Average of 3 replicates per trial.. Phase II confirmatory mutation assay (Experiment B4).
2AA = 2-aminoanthracene;
ne = Not tested in this strain of bacteria.
Data excerpted from pages of 41 & 42 of the study report.

III. CONCLUSION

Under the conditions of this study, IN-JSE76 was negative for bacterial mutagenic activity in non-activated and S9-activated test systems. This study is fully reliable (acceptable/guideline) and satisfies the requirements for a bacterial mutation assay (OPPTS 870-5100; OECD 471).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.10/05

Report: Snajdr, S.I. (2010); Cyantraniliprole (DPX-HGW86) technical: *In vitro* thyroid peroxidase inhibition. Dupont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-27123. September 9, 2010. MRID 48119979. Unpublished.

Guidelines: In house method
Deviations: Not applicable

GLP: Yes. Signed statements of GLP, Data Confidentiality, and Qaulity Assurnace were included in the report.

Executive summary:

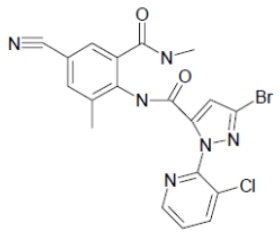
In thyroid hormone synthesis, thyroid peroxidase catalyzes the first two steps, oxidation of iodide to iodine and the iodination of tyrosine residues on thyroglobulin. Inhibition of thyroid peroxidase disrupts the homeostasis of the hypothalamic-pituitary-thyroid axis. This study (MRID 48119979) was designed to evaluate the ability of cyantraniliprole to inhibit thyroid peroxidase activity *in vitro* using thyroid preparations from the Yucatan pig (microswine). Cyantraniliprole concentrations ranging from 2 to 400 μM were tested; the maximum concentration was the level where limit of solubility was present in the assay system. Propylthiouracil (PTU), a known thyroid peroxidase inhibitor, was the positive control. The result showed that for PTU, the concentration that caused a 50% reduction in enzyme activity (IC_{50}) was 7.3 μM. In contrast cyantraniliprole did not cause inhibition of thyroid peroxidase at any concentration tested; therefore, an IC_{50} value for cyantraniliprole was unable to be determined. Under the conditions of this *in vitro* study, cyantraniliprole did not inhibit thyroid peroxidase.

This study is reliable and provides useful information on the effect of cyantraniliprole on the thyroid peroxidase derived from Yucatan pig (microswine).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
1H-pyrozole-5-carboxamide, 3-bromo-1-1(3-chloro-2-Pyridinyl)-N-[4cyano-2-methyl-6-[(methylamino)-carbonyl]-phenyl]



Lot/Batch #:	HGW86-230
Purity:	94.5%
Description:	Off-white solid
CAS #	736994-63-1
Stability of test compound:	The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed.

2. Control materials

Positive control:	Propylthiouracil (PTU) in 10% DMSO

3. Test system

Thyroid peroxidase preparation prepared from the thyroid gland of a 7-month old untreated microswine (Yucatan Pig). The thyroid gland was received from Sinclair Research Center, Inc. (Columbia, MO, USA).

4. Test compound concentrations used

0, 2, 5, 10, 20, 50, 100, 200, and 400 μM

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

July 24, 2009 to July 24, 2009

2. Methods

Cyantraniliprole was evaluated *in vitro* for its ability to inhibit the peroxidase catalyzed iodination of thyroglobulin using hydrogen peroxide as the substrate. A colorimetric assay was used to measure the activity of the peroxidase by measuring the conversion of guaiacol to a colored oxidation product, tetraguaiacol.

The microswine thyroid was dissected and trimmed free of fat and parathyroids by Sinclair Research Center, Inc., flash frozen in liquid nitrogen and maintained between -60 and -80°C until shipped frozen on dry ice to DuPont Haskell. On receipt, the thyroid was stored between -60 and -80°C until homogenization.

The thyroid was homogenized in buffer (250 mg tissue/1 mL) containing 10 mM Tris-HCl (pH 7.2). The homogenate was centrifuged at 39000 × g for 45 minutes at 4°C, the pellet was resuspended in buffer containing 10 mM Tris-HCl and 0.5 M $CaCl_2$ (pH 7.2), and the pellet was subsequently recentrifuged at 39000 × g for 45 minutes at 4°C. The pellet was resuspended, aliquoted, and stored between -60 and -80°C until analyzed for peroxidase activity. Before use it was centrifuged at 600 × g for 15 minutes at 4°C to remove small chunks of membrane. The total protein of the thyroid peroxidase preparation was determined by the Biorad Bradford method. The protein concentration of the thyroid peroxidase preparation was 0.641 mg/mL and was not diluted further prior to use.

At the time of the assay, 60 µL of the thyroid peroxidase preparation was added to designated wells on a 96-well plate, containing 20 µL of diluted test substance, positive control or vehicle. To initiate the reaction, 120 µL of reaction mixture (10 mM Tris-HCl, 0.5 M $CaCl_2$, 13 mM guaiacol, and 0.3 mM hydrogen peroxide) was added to each well. The rate of guaiacol oxidation was measured by reading absorbance at 470 nm for 5 minutes on a Molecular Devices SpectraMax Plus 384 UV/Vis cuvette/microplate reader. All test substance concentrations were assayed in quadruplicate. Concentrations of the positive controls and the vehicle were assayed in triplicate. The rate of thyroid peroxidase activity was expressed as the "change in absorbance at 470 nm/mg protein-minute" and was used to determine the concentration that reduced enzyme activity by 50% (IC_{50}) for the test substance and positive control.

Cyantraniliprole was diluted in 100% DMSO and evaluated at final concentrations of 2, 5, 10, 20, 50, 100, 200, and 400 µM; the highest concentration tested was the limit of solubility in the assay system. The positive control, PTU in 100% DMSO, was used to verify the test system performance. The final concentration of DMSO in each well was 10%.

3. Statistics

Where applicable, IC_{50} values were calculated using Origin 7.0 (OriginLab Corp., Northampton, Massachusetts, U.S.A.).

II. RESULTS AND DISCUSSION

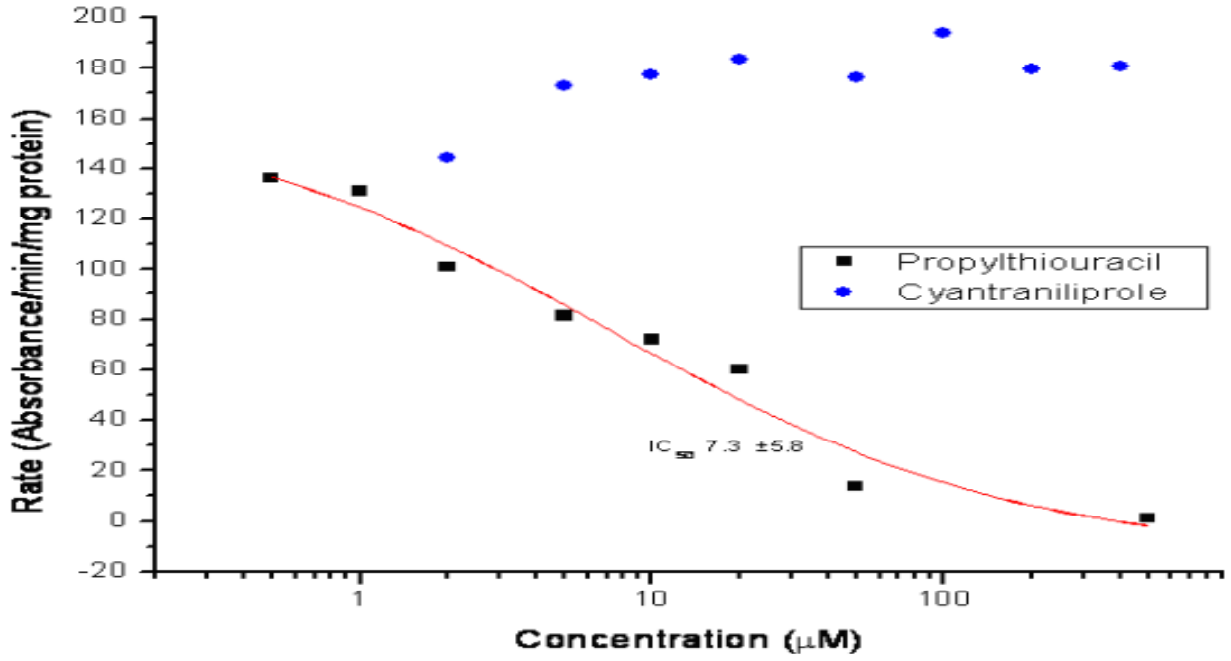
The results of the assays showed that cyantraniliprole, when tested up to a concentration of maximum solubility in the assay system, demonstrated no statistically significant difference in peroxidase activity between cyantraniliprole treated samples and the control (Table 1 & Figure 1). As a result, an IC_{50} value could not be determined. In comparison, the positive control, PTU, caused a dose-related decrease in thyroid peroxidase activity. The IC_{50} value was 7.3 µM.

Table 1. Summary of thyroid peroxidase activity *in vitro*

Cyantraniliprole		**Propylthiouracil (PTU**	
Final concentration (µM)	**Average activity (absorbance/min/mg protein)** [a]	**Final concentration (µM)**	**Average activity (absorbance/min/mg protein)** [a]
0	139.5 ± 36.3	0	139.5 ± 36.3
2	144.4 ± 27.3	0.5	136.3 ± 1.7
5	173.1 ± 36.7	1	131.0 ± 30.2
10	177.6 ± 26.8	2	101.2 ± 2.6
20	183.3 ± 18.6	5	81.3 ± 33.9
50	175.2 ± 15.1	10	72.1 ± 14.1
100	193.9 ± 17.5	20	60.1 ± 19.5
200	179.8 ± 33.1	50	13.8 ± 12.4
400	177.9 ± 32.3	500	1.0 ± 1.7
IC_{50}= Can not be established		IC_{50}=7.3 ±5.8 µM	

[a] n = 4 Data excerpted from pages 15 of the study report.

Figure 1. Thyroid Peroxidase Activity Inhibition
(Figure excerpted from page 17 of the study report)



III. CONCLUSIONS

Under the conditions of this study, cyantraniliprole, when tested up to a concentration of maximum solubility (400 μM) in the assay system, did not inhibit thyroid peroxidase *in vitro* using thyroid gland preparations from the Yucatan pig (microswine).

This study is reliable and provides useful information on the effect of cyantraniliprole on the thyroid peroxidase derived from Yucatan pig (microswine).

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.1/02	***In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay**
Report:	Wagner, V.O. VanDyke, M.R. (2009); Cyantraniliprole (DPX-HGW86) technical: Bacterial reverse mutation assay, DuPont-27160. BioReliance, Rockville, Maryland, USA **Report No.:** AC25MV.503.BTL. MRID 48119980
Dates of work:	26-Feburary-2009 to 25-March-2009
Guidelines:	**Guidelines:** OPPTS 870.5100 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: NO (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In independent trials of a reverse gene mutation assay (MRID 48119980), cyantraniliprole (97.7% Lot No. 9182-3B; HGW68-648) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) in two phases using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The dose levels tested were 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the lack of mutagenic potential of the test substance. The dose levels tested were 0, 50, 150, 500, 1500, and 5000 µg/plate. The highest dose level was set based on insolubility of the test substance and compatibility with the target cells. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a maximum concentration of approximately 150 mg/mL. After sonication for 35 minutes at 30°C, the test substance formed workable suspensions in DMSO from approximately 250 to 500 mg/mL and a soluble and clear solution at a maximum concentration of approximately 200 mg/mL.

Precipitate was observed at 5000 µg per plate in both trials. No appreciable toxicity was seen. The positive and vehicle controls fulfilled the requirements for a valid test. In the initial toxicity-mutation assay and in the confirmatory mutagenicity assay, no positive mutagenic response occurred.

Under the conditions of this study, cyantraniliprole was tested up to the limit dose and was negative for mutagenic activity in non-activated and S9-activated test systems.

The study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: 9182-3B
   Purity: 97.7
   Description: Off-white fine powder
   CAS # 736994-63-1
   Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.) Dosing formulations were adjusted to 100% purity using a correction factor of 1.0441
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)/50 µL

Positive control, non activation:

Positive control	Dose	Strain(s)
2-Nitrofluorene	1 µg/plate	TA98
Sodium azide	1 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-Aminoanthracene	1 µg/plate	TA98, TA1535, TA1537
	2 µg/plate	TA100
	10 µg/plate	WP2 *uvr*A

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2359
   Source: Moltox, Inc., Boone, NC
   Protein content: 35.4 mg/mL
   Source: Not applicable
   Characterisation: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition:
   Phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM
   $MgCl_{2:}$ 8 mM
   S9: 10% (v/v)

4. Test organisms

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

Exp. No. B1: (Initial Toxicity-Mutation Assay)	Concentrations of 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg cyantraniliprole/plate were evaluated in duplicate in the presence and absence of S9 activation.
Exp. No. B2: (Confirmatory Mutagenicity Assay)	Concentrations of 0, 50, 150, 500, 1500 and 5000 µg cyantraniliprole/ plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

26 February-2009 to 31-March-2009

2. Plate incorporation assay

This study consisted of 2 independent trials, Experiment No. B1 and Experiment No. B2. Experiment No. B1, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test substance were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537, and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. Experiment No. B2, the confirmatory mutagenicity test, was used to evaluate the mutagenic potential of the test substance. Five dose levels of test substance along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537, and WP2 *uvr*A on selective agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test substance, vehicle control and positive controls were plated in triplicate. One-half milliliter (0.5 mL) of S9 or Sham mix, 100 µL of tester strain (containing approximately 0.3×10^9 bacteria), and 50 µL of vehicle or test substance dilution were added to 2 mL of molten selective top agar (0.8% [w/v] agar and 0.5% NaCl [w/v]) supplemented with L-histidine, D-biotin, and L-tryptophan) at 45 ± 2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37 ± 2°C. Plates that were not counted immediately following the incubation period were stored at 2 to 8°C prior to evaluation and counting of revertant colonies.

The condition of the bacterial background lawn was evaluated for evidence of test substance toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity was scored relative to the concurrent vehicle control plates and recorded with the mean revertant count for the strain, condition and concentration. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity.

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

For the test substance to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test substance. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100, and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal. The performing laboratory provided historical control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on the solubility of the test substance and compatibility with the target cells. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (95.3, 86.3, and 93.5% of their respective targets). The stability analysis of the formulations found the test substance to be stable in DMSO at room temperature for the period of dosing. No test substance was detected in the vehicle control sample.

B. MUTATION ASSAYS

Compound precipitation was observed in both trials at 5000 μg per plate. No appreciable toxicity was observed in either trial as is evidenced by a reduction of the microcolony background lawns and/or by a concentration-related decrease in mean revertants per plate. All positive controls exhibited more than a 3-fold increase in mean revertants over the respective mean of the vehicle controls. For all strains except TA1535 and TA1537, no test substance concentration produced a mean 2 times greater than the mean of its respective vehicle control. For strains TA1535 and TA1537, no test substance concentration produced a mean 3 times greater than the mean of its respective vehicle control. There was no concentration-related increase in the mean revertants per plate in any strain in either trial (Tables 1 and 2).

Controls: The positive and vehicle controls fulfilled the requirements for a valid test.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 471(1997)

C. CONCLUSIONS: Cyantraniliprole was negative for mutagenic activity up to precipitating concentrations in all strains in both trials in the presence and absence of S9-activation in the *in vitro* bacterial gene mutation assay. There were no treatment-related increases in the mean number of revertants/plate in any strain (+/-S9). The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

Accordingly, Cyantraniliprole is negative in this test system in a well-done study.

Table 1
Summary of average revertants/plate without activation of Cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2
Cyantraniliprole	0	11[a]	17[b]	168	163	15	15	9	8	31	36
	1.5	11	ne	157	ne	14	ne	6	ne	32	ne
	5.0	10	ne	168	ne	16	ne	6	ne	25	ne
	15	14	ne	129	ne	8	ne	5	ne	33	ne
	50	12	16	175	177	15	16	6	8	29	29
	150	11	23	182	188	17	20	7	10	28	33
	500	14	17	141	167	15	23	6	11	26	34
	1500	12	20	155	191	16	25	7	8	29	24
	5000	11	20	154	186	12	29	8	9	16	34
2NF	1	209	197	ne	ne	ne	ne	ne	ne	ne	ne
NAAZ	1	ne	ne	681	550	453	373	ne	ne	ne	ne
9AA	75	ne	ne	ne	ne	ne	ne	1877	2198	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	407	355

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

2NF = 2-nitrofluorene; NAAZ = Sodium azide; 9AA = 9-aminoacridine; MMS = Methyl methanesulfonate;

ne = Not evaluated

Data were derived from Tables 21 and 22, pp. 41 and 42, MRID 48119980.

Table 2
Summary of average revertants/plate with activation of Cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2
Cyantraniliprole	0	24[a]	32[b]	122	180	15	18	9	7	34	34
	1.5	19	ne	120	ne	8	ne	9	ne	35	ne
	5.0	23	ne	99	ne	14	ne	5	ne	33	ne
	15	15	ne	114	ne	14	ne	4	ne	40	ne
	50	17	31	111	175	15	16	8	7	37	36
	150	20	31	108	220	13	16	8	8	22	40
	500	17	27	107	187	13	14	6	7	23	31
	1500	17	27	124	219	14	18	6	9	23	31
	5000	21	23	104	210	13	23	6	9	26	30
2AA	1	414	520	ne	ne	85	101	59	79	ne	ne
2AA	2	ne	ne	951	1367	ne	ne	ne	ne	ne	ne
2AA	10	ne	ne	ne	ne	ne	ne	ne	ne	293	278

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

2AA= 2-aminoanthracene

ne = not evaluated

Data were derived from Tables 21 and 22, pp. 41 and 42, MRID 48119980.

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Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIA 5.8/14

Report: Wagner III, V.O., VanDyke, M.R. (2009b); IN-N5M09: Bacterial reverse mutation assay. BioReliance, Rockville, Maryland, USA. Laboratory Report No.: AC29WT.503.BTL. DuPont Report No. DuPont-28800. September 24, 2009. MRID 48119982. Unpublished.

Guidelines: OPPTS 870-5100 (1998)
EEC 2000/32/EC, Annex 4D-B13/14 No. L136
OECD 471 (1998)
JMAFF 12 Nousan 8147 Guideline No. 2-1-19-1 (2000 and later revisions)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In a bacterial reverse mutation assay (MRID 48119982), IN-N5M09 (99.9% purity) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and in *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) in 2 trials using standard plate incorporation methods. The test substance was administered as a solution with propylene glycol as solvent. Based on the results of an initial trial toxicity-mutation assay (Trial I) with nominal concentrations of 1.5, 5.0, 15, 50, 150, 500 1500, and 5000 μg/plate, the confirmatory mutagenicity assay (Trial II) employed the following concentrations: 50, 150, 500, 1500 and 5000 μg/mL. Positive controls were: 2-nitrofluorene, 9-aminoacridine, methyl methanesulfonate, and 2-aminoanthracene.

The number of revertants at all concentrations of the test substance was comparable to concurrent controls in Trials I and II both with and without S9 activation. The positive controls produced expected increases in revertants. **Under the conditions of this study, IN-N5M09 was negative for bacterial mutation in non-activated and S9-activated test systems.**

This study is fully reliable (acceptable/guideline) and satisfies the requirements for bacterial reverse mutation assay (OPPTS 870-5100; OECD 471).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-N5M09 (metabolite of cyantraniliprole)
   Lot/Batch #: N5M09-003
   Purity: 99.9%
   Description: Yellow powder
   CAS # Not available
   Stability of test compound: Based on the expiration date of 30 October 2011 the test substance was considered stable for the purpose of this study.
2. Control materials
   Solvent control: Propylene glycol

Positive control, non activation:

Positive control	Dose	Strain(s)
2-nitrofluorene	1 µg/plate	TA98
sodium azide	1 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-aminoanthracene	1 µg/plate	TA98, TA1535, TA1537
	2 µg/plate	TA100
	10 µg/plate	WP2 *uvr*A

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (induced with 500 mg/kg Aroclor 1254)
   Lot number: 2448
   Source: Moltox, Inc., Boone, NC
   Protein content: 37.3 mg/mL
   Source: Not specified
   Characterisation: The metabolic activation ability of the S9 was characterised using varying S9 and positive control concentrations.
   S9 mix composition:
   Sodium phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM

$MgCl_2$:	8 mM
S9:	10% (v/v)

4. Test organisms

   Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rf a mutation, R factor).

5. Test concentrations for plate incorporation assay

Trial 1:	Concentrations of 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg IN-N5M09/plate were evaluated in duplicate in the presence and absence of S9 activation.
Trial 2:	Concentrations of 50, 150, 500, 1500, and 5000 μg IN-N5M09/plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   July 10, 2009 to August 22, 2009

2. Plate incorporation assay

   This study consisted of 2 independent trials that assessed test substance mutagenicity. The initial toxicity-mutation assay was used to establish the dose-range for the confirmatory mutagenicity evaluation. Vehicle control, positive controls, and eight dose levels of the test substance were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, and TA1537 and WP2*uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. The confirmatory mutagenicity assay was used to evaluate the mutagenic potential of the test substance. Five dose levels of test substance along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, and TA1537 and WP2*uvr*A on selective agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test substance, vehicle control and positive controls were plated in triplicate. Treatments with the exogenous metabolic activation system were conducted by adding 0.1 mL of negative (vehicle) control or test substance solution or 50 μL of the positive control substances, 0.5 mL of metabolic activation system, and 0.1 mL of an overnight culture containing approximately 1×10^9 bacteria/mL (the range was 0.8 to 4.1×10^9) to approximately 2 mL of top agar (0.8% [w/v] agar and 0.5% NaCl) containing 0.05 mM L-histidine, D-biotin, and L-tryptophan. These components were briefly mixed and poured onto a Vogel-Bonner minimal medium E agar plate. Treatments in the absence of the metabolic activation system were the same as those in the presence of the exogenous metabolic activation system with the exception that 0.5 mL of sterile buffer was used as a replacement for the volume of the exogenous metabolic activation system. After pouring onto the surface of minimal glucose agar

plates, the top agar was allowed time to solidify, and the individually labeled plates were inverted and incubated at approximately 37°C for approximately 48 to 72 hours. Plates that were not counted immediately were refrigerated at approximately 4°C (±3°C) prior to evaluation and counting of revertant colonies.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Toxicity was scored relative to the concurrent negative (vehicle) control plates and recorded with the mean revertant count for the strain, condition and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter or entirely by hand

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

For the test substance to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test substance. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100, and WP2 uvrA were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Samples of the 0.50, 5.0, and 50 mg/mL dosing formulations were shipped to the Sponsor for analysis. All treatment preparations were found to be stable in the vehicle during the dosing period. Concentration analysis indicated that the actual mean concentrations of the analyzed dose levels were 68.2, 70.6, and 71.6% of their respective targets with RSDs (relative standard deviations) of 11, 4, and 15%, respectively. No test substance was detected in the vehicle control sample. Although the actual concentrations of some of the analyzed dose levels were lower than expected and the formulations were not as homogeneous as desired, the study is considered valid since precipitate was observed at the top two to four dose levels in each assay, indicating that the test system was saturated with the test

substance. Therefore, it was concluded that the analytical results and the observed differences from nominal concentrations did not adversely impact the integrity of the data or the validity of the study conclusion.

B. MUTATION ASSAYS

A solubility test was conducted to determine the vehicle. The test was conducted using water, dimethyl sulfoxide (DMSO), ethanol (EtOH), acetone, dimethyl formamide (DMF), tetrahydrofuran (THF), propylene glycol, polyethylene glycol, and methanol to determine the solvent, selected in order of preference, that permitted preparation of the highest soluble or workable stock concentration up to 50 mg/mL for aqueous solvents and up to 500 mg/mL for organic solvents.

Propylene glycol was selected as the vehicle of choice based on the ability of the test substance to form a workable suspension in propylene glycol at approximately 50 mg/mL, the insolubility of the test substance in numerous other solvents or vehicle and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 5000 μg per plate; this dose was achieved using a concentration of 50 mg/mL and a 100 μL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg per plate. The test substance formed workable suspensions in propylene glycol from 0.50 to 50 mg/mL, soluble but cloudy solutions from 0.050 to 0.15 mg/mL and a soluble and clear solution at 0.015 mg/mL. Precipitate was observed beginning at 150 or 500 μg/plate. No appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose tested in the confirmatory mutagenicity assay was 5000 μg/plate. The summary of the results of the initial toxicity-mutation test (Trial I) and the confirmatory mutagenicity assay (Trail II) in either the presence or absence of S9 activation are presented in Tables 1 and 2,

In the initial toxicity-mutation assay (Trial I), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

In the confirmatory mutagenicity assay (Trail II), the dose levels tested were 50, 150, 500, 1500, and 5000 μg per plate. Precipitate was observed beginning at 500 or 1500 μg per plate. No appreciable toxicity was observed. However, no positive mutagenic responses were observed with any of the tester strains in either the presence or S9 absence of S9 activation.

All positive controls produced expected increases in revertants in the test system.

Table 1. Summary of average revertants/plate without activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I [a]	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-N5M09	0	19	32	113	89	11	9	6	5	27	27
	1.5	15	ne	107	ne	12	ne	7	ne	39	ne
	5	14		125		12		5		43	
	15	17		99		11		7		35	
	50	10	19	73	80	12	11	5	4	34	34
	150	14	30	108	78	9	8	7	5	26	27
	500	15	25	105	71	10	8	6	3	26	31
	1500	15	25	78	70	8	5	3	3	32	21
	5000	14	27	66	55	9	6	7	3	20	23
NAAZ	1	ne		516	382	494	323	ne			
9AAD	75	ne						1231	652	ne	
2NF	1	239	256	ne							
MMS	1000	ne								443	507

[a] Average of 2 replicates per trial ne = Not evaluated

2NF = 2-nitrofluorene; NAAZ = Sodium azide; 9AAD = 9-aminoacridine; MMS = Methylmethanesulfonate

Data excerpted from Table 1 in pages 20-21 (Trail I);Table 3 in pages 24 & 25 (Trail II) of the study report.

Table 2. Summary of average revertants/plate with activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I [a]	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-N5M09	0	16	32	114	82	12	7	4	6	29	33
	1.5	23	ne	130	ne	15	ne	6	ne	42	ne
	5	12		145		6		4		37	
	15	18		109		9		3		28	
	50	21	26	155	78	8	10	4	4	27	27
	150	18	35	160	80	11	9	4	6	30	28
	500	19	23	137	78	9	8	5	5	22	29
	1500	14	24	160	80	11	5	4	3	28	33
	5000	18	26	130	80	11	8	3	5	25	26
2AA	1	707	749	ne		74	82	82	76	ne	
	2	ne		1316	1219	ne					
	10	ne								333	420

[a] Average of 2 replicates per trial. Ne = Not evaluated. 2AA =2-aminoanthracene.

Data excerpted from Table 2 in pages 22 & 23 (Trail I), Table 4 in pages 26 & 27 (Trail II) of the study report.

III. CONCLUSION

IN-N5M09 was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.8.01

Report: Anand, S.S. (2010); IN-JSE76: Repeated-dose oral toxicity 28-day feeding study in rats. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-28842. May 28, 2010. MRID 48119983. Unpublished.

IIA 5.8/09

Report: Mawn, M.P. (2011); IN-JSE76: Repeated-dose oral toxicity 28-day feeding study 28842; Supplement No. 1.May 28, 2010. MRID 48204874. Unpublished.

Guidelines: OPPTS 870.3050 (2000)
OECD 407 (2008), EEC Method B.
7 Directive 96/54/EC (1996)
Deviations: None

GLP: Yes Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 28-day feeding study (MRID 48119983), IN-JSE76 (a metabolite of cyantraniliprole) was administered to male and female Crl:CD®(SD) rats (10 animals/sex/concentration) at concentrations of 0, 100, 400, 3000, and 20000 ppm (males: 0, 7, 29, 212, and 1445 mg/kg bw/day; females: 0, 8, 31, 232, and 1471 mg/kg bw/day). The following parameters were evaluated: body weight, body weight gain, food consumption, food efficiency, clinical signs, serum thyroid hormone levels, hepatic microsomal and peroxisomal enzymes (cytochrome P450, ß-oxidation and UDP-glucuronyltransferase), gross pathology, organ weights, haematology, clinical chemistry, urinalysis, and histopathology. Blood samples were also collected on test days 23 (male) and 24 (female) of the study. The plasma samples were analyzed to quantify the concentration of parent compound, IN-JSE76, and possible metabolites, IN-K5A78, IN-K5A79, and IN-PLT97 (MRID 48204874).

IN-JSE76 did not produce any treatment-related effects on the following measurements: survival, clinical or ophthalmological observations, body weight, food consumptions, most clinical pathology parameters (haematology, clinical chemistry, coagulation, urinalysis), or on gross pathology. At 20,000 ppm, IN-JSE76 produced a markedly drop in total bile acid in both males and female rats; the toxicological significance of this reduction was not clear because other associated changes were not found. There were no organ weight or anatomic pathology finding associated with treatment. In male rats, serum T_4 levels were statistically significantly decreased at dietary levels of 400, 3000, and 20000 ppm (19%. 30%,and 21%, respectively). Although not significant, there were also corresponding increases in TSH levels at 3000 and 20000 ppm (16% & 46%, respectively). These changes were considered to be test substance related, but the

adversity of this effect was doubtful when the all the results of this study were considered. Particularly there was a lack of treatment related changes in corresponding organ weight (spicifically thyroid or liver), microscopic pathology (thyroid or liver), or hepatic total cytochrome P450 or UDP-glucuronyltransferase activity in males or females.

The plasma concentrations of IN-JSE76 were approximately linear with respect to dose in both male and female rats over the range of doses tested. There was no sex difference in the plasma concentration of IN-JSE76. The only targeted metabolite that had quantifiable levels in plasma was IN-K5A78, which was reported for the highest dose group in females and in the two highest dose groups in males.

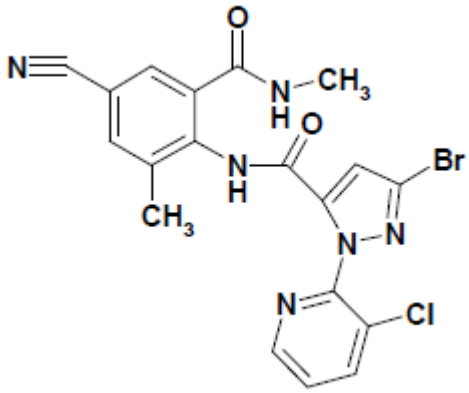
When all the results of this study were considered together, IN-JSE76 did not produced adverse effects at the highest concentration tested. The NOAEL was 20,000 ppm (males: 1445 mg/kg bw/day; females: 1471 mg/kg bw/day); LOAEL was not established.

This study is fully reliable (acceptable/guideline) meets the requirements for a 28-day oral toxicity study (OPPTS 870.3050; OECD 407).

MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole technical 3-Bromo-*N*-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carboxamide (Alternative IUPAC name)



	Lot/Batch #:	HGW86-014
	Purity:	97.8%
	Description:	Solid
	CAS #	Not available
	Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 22 days at room temperature, was distributed homogeneously and was present at targeted concentrations in the feed.
2.	Vehicle and/or positive control:	Untreated diet
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)
	Age at dosing:	Approximately 8 weeks old
	Weight at dosing:	228–285 g for males; 170–213 g for females
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	6 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

4. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	At least 10 air changes/hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experiment start/completion:

September 08, 2009 to May 28, 2010

In-life initiated/completed

September 08, 2009 to October 09, 2009

2. Animal assignment and treatment

Five groups of 10 animals/sex/concentration were administered concentrations of 0, 100, 400, 3000, and 20000 ppm IN-JSE76 in feed daily for approximately 28 days. (Table1). Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (NRC 1996).

Table 1. Study Design

Conc. in diet (ppm)	No./ group	Mean daily intakes mg/kg bw[a]	
0 (control)	10	0 (control)	0 (control)
100	10	7	8
400	10	29	31
3000	10	212	232
20000	10	1445	1471

[a]: Data excerpted from compound intake summary pages 43 of the report.

3. Diet preparation and analysis

The test substance was added directly to the rodent diet and thoroughly mixed a period of time that, by experience or pretest determination, was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. All diets were prepared up to established stability and refrigerated until used. The stability, homogeneity, and concentration of IN-JSE76 in the dietary mixtures were checked by analysis using HPLC at the beginning and end of study. The test substance was at target concentrations (98.5 ± 3.4% of nominal), homogeneously mixed in the feed, and was stable for up to 22 days.

4. Statistics

Table 2. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, organ weight, clinical pathology[a], organ weight, hormone levels, and biochemical evaluation	Levene's test for homogeneity and Shapiro-Wilk test[b] for normality	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.10, 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, is specific gravity was reported as >1.083, 1.083 was used for any calculations performed with that data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS

1. Observations

Animals were observed at least twice daily for clinical signs, mortality, and morbidity and weekly for clinical signs of toxicity.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology

Blood and urine samples were collected from all surviving animals after approximately 28 days of exposure to test or control diets for clinical pathology evaluation (haematology, clinical chemistry, coagulation, and urinalysis). At sacrifice, bone marrow was collected. Bone marrow analysis was not necessary to support experimental findings. The following parameters were examined.

Hematology

red blood cell count
hemoglobin
red cell distribution width
absolute reticulocyte count

hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular (cell) hemoglobin concentration	microscopic blood smear examination
prothrombin time	activated partial thromboplastin time

Clinical Chemistry

aspartate aminotransferase	glucose
alanine aminotransferase	total protein
sorbitol dehydrogenase	albumin
alkaline phosphatase	globulin
total bilirubin	calcium
urea nitrogen	inorganic phosphorus
creatinine	sodium
cholesterol	potassium
triglycerides	chloride
total bile acids	

Urinalysis

quality	ketone
color	bilirubin
clarity	blood
volume	urobilinogen
specific gravity	protein
pH	microscopic urine sediment
glucose	examination

6. Thyroid hormone analysis

   Blood (~1 mL) was collected, by orbital sinus bleeding of male and female rats from each group, on test days 23 (males) and 24 (females). Serum was prepared and stored frozen until analysed for T_4, T_3, and TSH concentrations.

7. Biochemical analysis

   At necropsy, a portion of the liver from each of these animals was homogenised and hepatic microsomes and peroxisomes prepared using differential centrifugation. The pellets were resuspended in homogenisation buffer and stored frozen until analysed for UDP-GT, peroxisomal β-oxidation, and total cytochrome P-450 content. The protein content of the microsomes and peroxisomes was determined before and after analysis by the Biorad method.

8. Plasma concentration

   Blood (unfasted) was collected on test days 23 (males) and 24 (females) for

determination of plasma concentration of the test substance and selected metabolites. Samples were evaluated by liquid chromatography-mass spectroscopy (LC-MS).

9. Necropsy and anatomic pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (20000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically.

Table 3. Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/ histopathologic evaluation conducted[a]
Liver	X	X
Kidneys	X	X
Heart	X	X
Spleen	X	X
Thymus	X	X
Thyroid gland	X	X
Adrenal glands	X	X
Brain (3 sections)	X	X
Males: Testes	X	X
Epididymides	X	X
Prostate (with seminal vesicles and coagulating glands)	X	X
Females: Ovaries (with oviducts)	X	X
Uterus (with cervix)	X	X
Esophagus		X
Stomach		X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Urinary bladder		X
Lungs		X
Trachea		X
Nose (4 sections)		X
Larynx/pharynx		X
Aorta		X
Mandibular lymph node		X
Mesenteric lymph node		X
Bone marrow		X
Peyer's patches		X
Pituitary gland		X
Parathyroid glands		X
Spinal cord (3 levels)		X
Sciatic nerve		X
Eyes (with retina and optic nerve)		X

Table 3. Organs/tissues collected for pathological examination		
Organ	**Organs weighed**	**Microscopic/ histopathologic evaluation conducted[a]**
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Females: Vagina		X
Mammary glands		X
Skin		X

[a] Only tissues from animals in the highest dose and control groups were evaluated

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No test substance-related clinical signs of toxicity were observed at any dietary concentration in either males or females.

2. Mortality

   Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains. Final (test day 28) body weights in male and female 20000 ppm groups was 97% and 98% of control respectively (neither statistically significant). Mean overall (test days 0-28) body weight gain in this same group was 94% and 87% of control, respectively (neither statistically significant).

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no test substance-related effects on food consumption or food efficiency. Mean overall (test days 0-28) food consumption in the male and female 20000 ppm male and female groups was 100% and 97% of controls, respectively (neither statistically significant). Mean food efficiency in these same group was 94% and 88% of control, respectively (neither statistically significant).

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related ophthalmological observations were noted at any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

   No substance-related changes in haematology parameters in male or female rats.

2. Clinical chemistry

There was a markedly drop in total bile acid in 20,000 ppm males and females and demonstrated a statistically significant difference from the controls. This appeared to be treatment related. However, the toxicological significance of this reduction is not clear because no other associated changes were seen.

- Activated partial thromboplastin time was minimally lower in female rats dosed with 3000 ppm; it was not dose or treatment related

- Blood urea nitrogen was minimally lower in only female rats dosed with 400, 3000, or 20000 ppm. However, the differences did not occur in a clear dose-response pattern; there were no changes in other tests of renal function (*i.e.*, specific gravity, creatinine).

Table Summary of relevant clinical pathology parameters

ppm	0	100	400	3,000	20,000
	Males				
APTT[a] (sec)	15.1±1.3	15.3±1.4	15.1±1.4	15.5±1.0	15.0±0.9
BUN (mg/dL)	11±1	13±3	13±2	12±2	11±1
TBA (µmole/L)	22.7±22.0	52.2±80.0	25.2±25.3	23.9±29.6	4.7±1.6*(↓79%)
	Females				
APTT[a] (sec)	15.4±1.8	14.4±1.0	15.0±1.3	13.7±0.5*	14.4±1.0
BUN (mg/dL)	17±2	15±2	15±1* (↓12%)	14±2*(↓18%)	14±2* (↓18%)
TBA (µmole/L)	40.3±54.1	14.7±8.9	43.5±76.8	12.5±4.5	8.8±2.4*(↓78%)

* Statistically significant ($p<0.05$) by Dunn's test
[a] ATT = attivated partial thomboplastin time; BUN = blood urea nitrogen; TBA = total bile acid
Data excerpted from pages 51-55 of the report.

3. Urinalysis

There were no treatment-related changes in group mean urinalyses parameters in male or female rats.

F. THYROID HORMONE ANALYSIS

T_4, T_3, or TSH levels in male or female rats are summarized in Table 4.

Table 4. Summary of hormone analyses					
Ppm	**0**	**100**	**400**	**3,000**	**20,000**
Males					
T_3 (ng/mL)	62.5±5.8	61.1±10.0	64.5±10.7	57.8±6.2 (↓8%)	57.8±6.9 (↓8%)
T_4 (µg/mL)	5.3±0.5	5.1±0.9	4.3±0.6* (↓19%)	3.7±0.5*(↓30%)	4.2±0.6*(↓21%)
TSH (ng/mL)	5.7±3.7	5.0±2.3	5.1±1.7	6.6±3.1(↑16%)	8.3±4.0(↑46%)
Females					
T_3 (ng/mL)	66.9±5.1	70.1±7.6	70.6±8.9	69.1±8.5	68.7±7.5
T_4 (µg/mL)	2.9±0.9	3.3±0.8	2.4±0.8	2.3±0.7	3.0±0.8
TSH (ng/mL)	9.3±5.1	8.3±3.6	8.3±2.0	8.8±5.3	9.4±3.3

* Statistically significant ($p<0.05$)

Data excerpted from pages 78-79 of the report

In male rats, serum T_4 levels were statistically significantly decreased at dietary levels of 400, 3000, and 20000 ppm IN-JSE76 (19, 30, and 21%, respectively). Although not significant, there were also corresponding increases in TSH levels at 3000 and 20000 ppm (16 and 46%, respectively). The changes in serum T_4 levels and TSH levels in males were considered to be test substance related, but did not appear to be adverse due to the lack of corresponding organ weight, microscopic pathology, or enzyme activity changes. There was no test substance-related effect on serum T_3 in males and T_4, T_3, or TSH in females in any dietary concentration.

G. BIOCHEMICAL ANALYSIS

No test substance-induced alterations were observed in ß-oxidation activity, UDP-GT activity, or total cytochrome P-450 content in male or female rats (Table 5).

Table 5 . Summary of hepatic biochemical analyses					
ppm	**0**	**100**	**400**	**3,000**	**20,000**
Males					
ß-oxidation rate (nmole/mg-min)	3.2±0.6	3.1±0.7	3.2±0.7	3.1±0.8	3.8±0.8
Total cytochrome P450 content (nmole/mg protein)	0.60±0.08	0.60±0.11	0.59±0.11	0.54±0.17	0.64±0.07
UDP-glucoronyltrans-ferase rate (nmole/min-mg)	23.7±4.9	21.9±7.2	23.9±6.0	24.4±5.7	23.2±4.3

Table 5 . Summary of hepatic biochemical analyses					
ppm	**0**	**100**	**400**	**3,000**	**20,000**
Females					
ß-oxidation rate (nmole/mg-min)	4.9±1.2	4.3±0.6	4.4±0.8	5.3±1.0	5.3±0.8
Total cytochrome P450 content (nmole/mg protein)	0.50±0.12	0.46±0.06	0.47±0.07	0.48±0.12	0.55±0.09
UDP-glucoronyltrans-ferase rate (nmole/min-mg)	14.5±3.0	16.9±3.7	13.7±4.6	14.4±6.9	13.1±7.9

Data excerpted from pages 80-81 of the study report.

H. PLASMA CONCENTRATION

The plasma concentrations of IN-JSE76 were approximately linear with respect to dose in both male and female rats over the range of doses tested (Table 5). There was no sex difference in the plasma concentration of IN-JSE76. The only targeted metabolite that had quantifiable levels in plasma was IN-K5A78, which was reported for the highest dose group in females and in the two highest dose groups in males.

Table 6. Summary of plasma concentration of IN-J and other metabolites (ng/mL)					
Ppm	**0**	**100**	**400**	**3,000**	**20,000**
Femmles					
IN-JSE76	ND	<5.0±NA	6.86±1.46	46.8±19.0	442±172
IN-K5A78	ND	ND	ND	<5.0±NA	37.4±10.4
Males					
IN-JSE76	ND	<5.00	5.62±0.42	41.6±11.4	423±125
IN-K5A78	Va	ND	ND	7.95±NA[a]	25.6±7.6

[a]: Value for a single sample that was above the limit of quantitation of 5.00 ng/mL.
Data excerpted from pages 15-16 of the report

I. NECROPSY AND ANATOMIC PATHOLOGY

1. Organ weight

No test substance-related changes in mean organ weights or organ weights relative to final body weight or brain weight were apparent at any dietary concentration in males or females.

2. Gross and microscopic pathology

No test substance-related gross lesions were observed at necropsy. There were no test substance-related microscopic findings in the male or female 20000-ppm groups.

III. CONCLUSION

Under the conditions of this study, IN-JSE76 did not produce any effects on the following parameters: survival, clinical or ophthalmological observations, body weight, food consumptions, any most clinical pathology parameters (haematology, clinical chemistry, coagulation, urinalysis), or on gross pathology. At 20,000 ppm, IN-JSE76 produced a markedly drop in total bile acid in both males and female rats; the toxicological significance of this reduction was not clear. There were no organ weight or anatomic pathology finding associated with treatment. In male rats, serum T_4 levels were statistically significantly decreased at dietary levels of 400, 3000, and 20000 ppm (19%. 30%,and 21%, respectively). Although not significant, there were also corresponding increases in TSH levels at 3000 and 20000 ppm (16% & 46%, respectively). These changes were considered to be test substance related, but the adversity of this effect was doubtful when the all the results of this study were considered. Particularly there was a lack of treatment related changes in corresponding organ weight (specifically thyroid or liver), microscopic pathology (thyroid or liver), or hepatic total cytochrome P450 or UDP-glucuronyltransferase activity in males or females.

The plasma concentrations of IN-JSE76 were approximately linear with respect to dose in both male and female rats over the range of doses tested. There was no sex difference in the plasma concentration of IN-JSE76. The only targeted metabolite that had quantifiable levels in plasma was IN-K5A78, which was reported for the highest dose group in females and in the two highest dose groups in males.

When all the results of this study were considered together, IN-JSE76 did not produced adverse effects at the highest concentration tested. The NOAEL was 20,000 ppm (males: 1445 mg/kg bw/day; females: 1471 mg/kg bw/day); LOAEL was not established.

This study is fully reliable (acceptable/guideline) meets the requirements for a 28-day oral toxicity study (OPPTS 870.3050; OECD 407).

Global Primary Reviewer: Whang Phang, PhD

IIA 5.10/04

Report: MacKenzie, S.A. (2010b); Cyantraniliprole (DPX-HGW86) technical: Adrenal mechanistic study 90-day feeding study in mice. DuPont Haskell Laboratories, Newark, Delaware, USA; Experimental Pathology Laboratories, Inc.; Laboratory for Advanced Electron and Light Optical Methods (LAELOM), Durham, North Carolina, Raleigh, North Carolina, USA. Laboratory Report No.: DuPont-29405. MRID 48119985. Unpublished.

Guidelines: Not applicable

Deviations: Not applicable

GLP: Yes. Signed statements of Good Laboratory Practice Compliance, Data Confidentiality, and Quality Assurance were included in the report.

Executive summary:

This study was performed to elucidate the mechanism of adrenal microvesivulaiton observed in the 90-day feeding study in mice with cyantraniliprole (MRID 48119943). Previously, an increase in the incidence of adrenal cortex microvesiculation was observed in male mice exposed to 50, 300, 1000, and 7000 ppm in the diet for 90 days. Presently, for this study, 2 groups of approximately 7 weeks old male Crl:CD1® mice (10/group) were fed control diet or diet containing 7000 ppm (1120 mg/kg bw/day) for 93 days. The batch of test material (DPX-HGW86-141 or cyantraniliprole) used in this study was the same test batch used in the previous 90-day mouse study. Body weights, food consumption, and detailed clinical observations were performed weekly and clinical observations were for overt signs of toxicity were conducted daily. After approximately 12 weeks of exposure, urine corticosterone, urine volume, and urine creatinine were measured in mice following an overnight collection of urine. After approximately 13 weeks (93 days) of exposure, mice were sacrificed and adrenal glands were weighed and collected. The right adrenal gland was evaluated microscopically from all mice. Left adrenal glands from 4 mice/group were evaluated by electron microscopy.

Under the conditions of this study, cyantraniliprole at 7000 ppm (1120 mg/kg/day) did not produce deaths, clinical signs of toxicity, adverse effects on body weight, food consumption, or food efficiency. No gross or microscopic pathology effects were attributed to test substance exposure. No adverse effects on adrenal cortical structure or function were found. Basal urinary corticosterone was comparable between the control and treated groups. The electron microscopy results showed that cyantraniliprole did not affect adrenal cortical cell structure. The finding of the microvesiculation of the adrenal cortex in male mice at ≥ 50 ppm in the 90-day study (MRID 48119943) was not duplicated in this study, where male mice were fed dietary concentration of 7000 ppm (1120 mg/kg/day) of cyantraniliprole for 93 days.

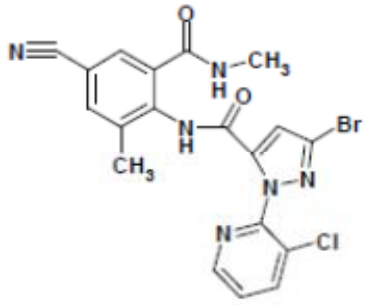
This study is considered reliable (acceptable/non-guideline) and provides some information concerning microvesiculation of the adrenal cortex seen in the 90-day feeding study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical

3-Bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide



Lot/Batch #:	HGW86-141
Purity:	91.5%
Description:	Off-white solid
CAS #	736994-63-1
Stability of test compound:	Analyses confirmed that the test material was stable in the feed, distributed uniformly, and present at the target concentrations.

2. Vehicle and/or positive control: Untreated diet

3. Test animals

Species:	Mouse
Strain:	Crl:CD-1®
Age at dosing:	Approximately 7 weeks old
Weight at dosing:	28.1–32.0 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	8 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards with nylabones as enrichment.

4. Environmental conditions

Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. Experiment start/completion

   11-November-2009 to 21-June-2010

   In-life initiated/completed

   11-November-2009 to 12-February-2010

2. Animal assignment and treatment

   Two groups of 10 males/concentration were administered 0 or 7000 ppm cyantraniliprole in feed daily for 93 days (Table 1). Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means. The negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (NRC 1996).

Table 1. Study design

Group no.	No. of male mice/group	Conc. in diet (ppm)[a]	Mean daily intakes[b] mg/kg bw
1	10	0	0
2	10	7000	1120

[a] Weight/weight concentration of test substance (adjusted for sponsor-supplied 91.5% purity of active ingredient).
[b] Data excerpted from page 29 of the report

3. Diet preparation and analysis

   The test substance was added to the rodent diet and thoroughly mixed for a period of time that, by experience, was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. Diets were prepared approximately every other week and refrigerated until used. The homogeneity (beginning of the study only) and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC at the beginning, in the middle, and at the end of the study. The test substance was at target concentrations (±6.6%), and homogeneous (RSD = 1%) throughout the feed. Stability of the test substance up to 14 days at room temperature or up to 21 days refrigerated was demonstrated in a previously conducted 90-day study. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics. The statistical methods employed in this study are shown in Table 2.

Table 1. Statistics

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, clinical pathology[a], corticosterone levels, organ weight	Levene's test and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed with Dunnett's test	Kruskal-Wallis test followed with Dunn's test

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for calculations performed with that bilirubin data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS

1. Observations

   Animals were observed at least once daily for mortality and morbidity and examined weekly for clinical signs of toxicity.

2. Body weights

   All animals were weighed once per week and on the day of sacrifice.

3. Food consumption, food efficiency and daily intake

   Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Biochemistry/mechanistic parameters

   Urine was collected overnight from the mice approximately one week prior to scheduled sacrifice to evaluate baseline (basal) urine corticosterone. The following urine parameters were measured: creatinine, corticosterone concentration, and volume.

5. Sacrifice and pathology

   After approximately 93 days on study, the surviving male mice from each exposure level were sacrificed and necropsied. Gross examinations were performed on all mice and final body weights were recorded. The adrenal glands were weighed and placed in the appropriate fixative. The right adrenal gland was processed to slides and examined microscopically by a veterinary pathologist. The left adrenal gland of each of 4 male control and 7000 ppm mice was evaluated by transmission electron microscopy.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   No clinical signs were attributed to test substance exposure.

2. Mortality

   All animals survived to scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN

No adverse effects on body weight or body weight gain were observed in treated mice.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

No adverse effects on food consumption or food efficiency were observed in treated mice.

D. MECHANISTIC PARAMETERS

No adverse or statistically significant effects on urinary corticosterone (absolute or per ml urine) and corticosterone/creatine ratio were observed in treated mice.

Table 3. Summary of urine corticosterone levels in treated males

ppm	0 ppm	7000 ppm
Corticosterone (ng/mL)	39±18	57±19
Corticosterone (ng)	39±19	28±15
Corticosterone: creatinine ratio (%)	91±29	83±21

E. SACRIFICE AND ANATOMIC PATHOLOGY

1. Organ weight

   There were no test substance-related effects on absolute and relative (% body weight) adrenal gland weight.

2. Gross and microscopic pathology

   There were no test substance-related effects light microscopic examination of adrenal glands. With electron microscopic examination, the results showed minimal microvesiculation in the *zona fasciculata* layer of the adrenal cortex was present in both controls and 7000 ppm males, such that no distinction could be made that would indicate lipid vacuolation was related to exposure to cyantraniliprole. The increase in the incidence of microvesiculation in the adrenal cortex seen in the 90-day study (MRID 48119943) was not duplicated in this mechanistic study.

III. CONCLUSION

Under the conditions of this study, male mice fed 7000 ppm (1120 mg/kg/day) cyantraniliprole did not demonstrate an increase in the incidence of microvesiculation in the adrenal cortex and any changes urinary corticosterone level and corticosterone/creatine ratio.

This study is consider reliable and provides some information concerning microvesiculation of the adrenal cortex seen in the 90-day feeding study.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.5.1 Combined chronic oral toxicity/carcinogenicity study in rats (2-year)

IIA 5.5.1/01

Report: Craig, L. (2011); Cyantraniliprole technical (DPX-HGW86 commercial batch -412): Combined chronic toxicity/oncogenicity study 2-year feeding study in rats. MPI Research, Inc., Mattawan, Michigan, USA. Laboratory report No. 125-101. DuPont-26842. April 28, 2011. MRID 48122577. Unpublished

Guidelines: OECD Section 4 (Part 453) (1981 and 2009)
OPPTS 870.4300 (1998)
Commission Directive 88/302/EC Part B.33 (1988)
MAFF 12 Nousan 8147 (2000)

GLP: Yes OECD Principles of GLP, ENV/MC/CHEM (1998)
Singed and dated GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In a 2-year chronic toxicity/carcinogenicity study in rats (MRID 48122577), cyantraniliprole (97.0%; HGW86-412) was administered to groups (70 rats/sex/concentration) of Crl:CD®(SD) rats at dietary concentrations of 0, 20, 200, 2000, and 20000 ppm (males: 0, 0.8, 8.3, 84.8, and 906.6 mg/kg bw/day. females: 0, 1.1, 10.5, 106.6, and 1160.8 mg/kg bw/day). Ten rats/sex/concentration were sacrificed after one year for evaluation of chronic toxicity. All surviving rats were sacrificed after approximately 2 years on study. Body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, ophthalmology, organ weights, gross and microscopic pathology were evaluated according to the guidelines.

Under the condition of this study cyantranililprole did not affect survival, clinical or ophthalmological observations, or gross pathology. At 20000 ppm, small reductions in body weight, body weight gain, and food efficiency were observed primarily over the first 1-1.5 years of dietary exposure. Three male rats in the 20000 ppm group had increases in liver enzyme levels at 12 months. These changes were associated with liver microscopic pathology (focal necrosis) in two of these three rats at the interim sacrifice, and with focal vacuolation observed at increased incidence at the end of the study. No other adverse effects were observed in any group on clinical pathology parameters, survival, or macroscopic pathology.

Test substance-related effects were observed in liver after one year and in liver and kidney (females) after two years. After two years, the incidence of foci of cellular alteration (clear cell, eosinophilic and basophilic) and focal vacuolation was increased in livers of males at ≥2000 ppm (variable statistical significance).

Increases in liver weight (variable statistical significance) were observed in males and females in the 2000 and 20000 ppm groups at the one-year interim sacrifice and in 20000 ppm males at the terminal sacrifice. The incidence of hepatocellular hypertrophy was increased in males and females at 2000 and 20000 ppm after one year and after two years (males only increased at

20000 ppm). These effects were considered to be non-adverse and secondary to enzyme induction. Considering all the data in this study, cyantraniliprole did not produce compound-related or dose-related increase in tumor incidence.

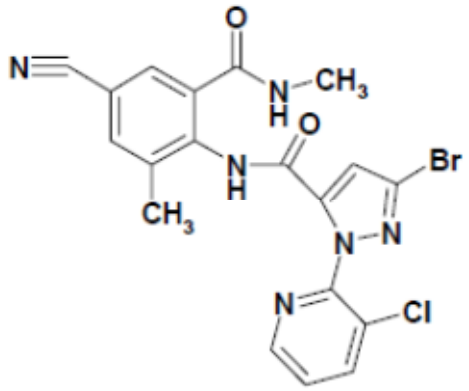
The no-observed-adverse-effect level (NOAEL) was 200 ppm (8.3 mg/kg bw/day) The LOAEL was 2000 ppm (84.8 mg/kg bw/day) based on microscopic liver pathology characterized by foci of cellular alteration (clear, eosinophilic, and basophilic) and focal vacuolation.

This study is classified as fully reliable (acceptable/guideline) and satisfied the guideline requirements (OPPTS 870.4300; OECD Section 4 (Part 453)) for a combined chronic oral toxicity/carcinogenicity study in rats.

I. MATERIALS AND METHODS

A MATERIALS

1. Test material: Cyantraniliprole technical
Synonym: 3-bromo-N-[-4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide.



Lot/Batch #:	9182-7 / HGW86-412;
Purity:	97.0%
Description:	White powder
CAS #:	736994-63-1
Stability of test compound:	Batches were prepared weekly and stored at room temperature until used. Reported to be stable in feed for at least 14 days at room temperature and present in the feed at targeted concentrations.

2. Vehicle and/or positive control: Untreated diet

3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)
Age at dosing:	Approximately 56 days old
Weight at dosing:	178-202 g for males; 151-177 g for females
Source:	Charles River Laboratories, Inc., Portage, MI
Acclimation period:	14 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	During the study, animals were pair-housed in solid bottom polybox cages. If an animal died, the cage-mate was housed individually. Animals were provided with Nylabones as environmental enrichment.

4 Environmental conditions

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Temperature:	22 ± 4°C
Humidity:	50 ± 20%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   December 3, 2008 to December 6, 2010

2. Animal assignment and treatment

   Five groups of 70 animals/sex/concentration were fed cyantraniliprole at concentrations of 0, 20, 200, 2000, and 20000 ppm. Ten animals/sex/concentration were sacrificed after one year for evaluation of chronic toxicity. The remaining animals were exposed to test diets for up to two years to evaluate oncogenic potential. The report indicated that dietary concentrations were selected based on previous rat studies where increases in liver and thyroid weights and in the incidence of hepatocellular and thyroid follicular cell hypertrophy were observed, with no effects on body weight or nutritional parameters or clinical pathology. Animals were assigned to dose groups by a standard block randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study design							
Males				**Females**			
Group no.	**No./ group[a]**	**Conc. in diet (ppm)[b]**	**Mean daily intakes[c] mg/kg bw**	**Group No.**	**No./ group**	**Conc. in diet (ppm)**	**Mean daily intakes mg/kg bw**
1	70	0 (control)	0 (control)	1	70	0 (control)	0 (control)
2	70	20	0.8	2	70	20	1.1
3	70	200	8.3	3	70	200	10.5
4	70	2000	84.8	4	70	2000	106.6
5	70	20000	906.6	5	70	20000	1160.8

[a] 10 rats/sex/group were designated as 12- month interim sacrifice.
[b] Weight/weight concentration of test substance, adjusted for purity
[c]

3. Diet preparation and analysis

The test substance was added to the rodent diet and thoroughly mixed for 20 minutes the first week and 10 minutes thereafter. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The stability, homogeneity and concentration of cyantraniliprole in the dietary mixtures were checked by analysis using HPLC/UV at study start. Analysis for concentration verification was also conducted approximately every 3 months. The test substance was at targeted concentrations ±12%, except for the 20 ppm diet which was >120% of nominal at weeks 66, 79, and 92. The diets were mixed homogeneously (≥15% RSD) throughout the feed and were stable (100.4-107.9% of nominal) for up to 14 days at room temperature.

4. Statistics (Table 2)

Table 2. Statistics employed in analyzing the results.	
Parameter	**Statistical Methods**
Body weight, body weight gain, food consumption, organ weight, haematology, clinical chemistry, coagulation	Group Pair-wise comparisons: Levene's test for homogeneity. If not significant, then Dunnett's test. If Levene's test was significant, then Welch's t-test with Bonferroni correction.
Leukocyte counts (total and differential)	Log transformation/ Group Pair-wise comparisons (Levene's/ANOVA-Dunnett's/Welch's)
Food efficiency, Urinalysis (volume, specific gravity, pH)	Rank transformation with Dunnett's test
Mortality data	Kaplan-Meier product-limit method
Microscopic pathology data (neoplastic and non-neoplastic)	Cochran-Armitage trend test Fisher's exact test

Significance was judged at $p < 0.05$ and < 0.01. Separate analyses were performed on the data collected for each sex.

C. METHODS

1. Observations

   Animals were observed at least twice daily for mortality and morbidity and examined for detailed clinical signs of toxicity weekly for the first 13 weeks then every other week thereafter.

2. Body weights

   All animals were weighed once per week. No body weight was recorded on the first day of dosing. Therefore, all body weight gains were calculated from the week -2 starting value.

3. Food consumption, food efficiency, and daily intake

   Food consumption was recorded for each animal weekly for the first 13 weeks then every other week thereafter. Food efficiency and daily intake (compound consumption) were calculated from food consumption and body weight data.

4. Ophthalmological examinations

   All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to their scheduled sacrifice at either 12 or 24 months.

5. Clinical pathology (hematology, clinical chemistry, urinalysis)

   Animals were fasted approximately 16 hours prior to sample collection. Blood and urine samples were collected from designated animals (10 animals/sex/group) at approximately 6 and 12 months after initiation of the study and analyzed for hematology, clinical chemistry, coagulation parameters, and urinalysis. Blood was collected from all surviving animals at 24 months and from animals euthanized *in extremis*, and evaluated for hematology (including WBC differential counts). Blood smears were prepared from blood samples (0.5 ml) collected from all surviving animals at 12 and 18 months, but they were not evaluated as no effects were observed at 24 months. The parameters examined are listed below:

Hematology

red blood cell count	red cell distribution width
hemoglobin	absolute reticulocyte count
hematocrit	platelet count
mean corpuscular (cell) volume	white blood cell count
mean corpuscular (cell) hemoglobin	differential white blood cell count
mean corpuscular (cell) hemoglobin concentration	microscopic blood smear examination
prothrombin time	activated partial thromboplastin time

Clinical Chemistry

aspartate aminotransferase	glucose
alanine aminotransferase	total protein
sorbitol dehydrogenase	albumin
alkaline phosphatase	globulin
gamma glutamyl transferase	calcium
urea nitrogen	inorganic phosphorus
creatinine	sodium
cholesterol	potassium
triglycerides	chloride
total bilirubin	

Urinalysis

quality	ketone
color and appearance	bilirubin
clarity	blood
volume	urobilinogen
specific gravity	protein
pH	microscopic urine sediment
glucose	examination

6. Sacrifice and pathology

At the one-year interim time point (10 animals/sex/group) and at study termination (all surviving animals), animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all animals. Organs that were weighed and collected are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (20000 ppm) and control (0 ppm) were processed and evaluated microscopically (Table 3). Microscopic evaluations were also conducted on the 20, 200, and 2000 ppm animals as the following: livers from animals scheduled for sacrifice (interim and terminal); livers from any animals that died prior to sacrifice; kidneys and non-glandular stomachs from the 24-month females; and thyroid glands from females of the interim and terminal sacrifice groups.

Table 3. Organs/tissues collected for pathological examination

Tissue	Organ weight taken	Collected and preserved	Microscopic examination	
			1, 5[a]	2-4[a]
Adrenal gland	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Epididymis	X	X	X	
Esophagus		X	X	
Eye (with optic nerve and retina)		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidney[a]	X	X	X	
Lacrimal gland, exorbital		X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver[b]	X	X	X	X
Lung with bronchi		X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (process females only)		X	X	
Nerve, sciatic		X	X	
Nose		X	X	
Ovary	X	X	X	
Oviducts		X	X	
Pancreas		X	X	
Peyer's patch		X	X	
Pharynx		X	X	
Pituitary		X	X	
Prostate		X	X	
Salivary gland, mandibular		X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Seminal vesicles		X	X	
Skeletal muscle, biceps femoris		X	X	
Skin		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, glandular		X	X	
Stomach, nonglandular[b]		X	X	

Table 3. Organs/tissues collected for pathological examination

Tissue	Organ weight taken	Collected and preserved	Microscopic examination	
			1, 5[a]	2-4[a]
Target Organs		X	X	X
Testis	X	X	X	
Thymus		X	X	
Thyroid gland (with parathyroid) [b]	X	X	X	
Tongue		X	X	
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix	X	X	X	
Vagina		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node		X	X	X

[a]: Test group. [b]: Designated as a target organs

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

   Treatment-related clinical signs of toxicity were not found in any dietary concentration.

2. Mortality

   No treatment--related effect on mortality was seen. The survival rates of females in 2000 and 20000 ppm were slightly higher. than control animals.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were slight decreases in mean body weight at various examined periods in the 20000 ppm males and females (Table 4); mean body weights were 6% and 9% lower than controls in males and females, respectively, at one year and 6% and 4%, respectively, at study termination. Body weight gains were 8% and 9% lower than controls in males and females, respectively, over one year, and 8% and 7%, respectively, over the two year period (Table 5). Mean body weight was lower in females at 2000 ppm over most of the first year of the study and body weight gain was significantly below control over the first 13 weeks of the study, but not over the entire first year. The slight reductions in mean body weight and body weight gain were found to be statistically significant at several weekly/biweekly measuring intervals.

Table 4. Body Weights (gm)					
Day	**0 ppm**	**20 ppm**	**200 ppm**	**2000 ppm**	**20000 ppm**
Males:					
Week -2	190.9±6.5	190.9±6.4	190.8±6.4	190.9±6.4	190.9±6.4
Week 13	576.5±55.9	564.7±58.6	578.1±53.7	556.8±44.5	543.7±43.2**(↓6%)
Week 52	765.8±90.3	781.8±106.5	768.5±101.7	758.2±91.0	718.7±71.2* (↓6%)
Week 103	782.8±83.3	793.9±143.2	761.6±103.5	762.6±69.6	737.4± 132.8(↓6%)
Females:					
Week -2	163.8±6.7	163.9±6.8	163.8±6.7	163.8±6.8	163.9±6.8
Week 13	315.2±29.3	304.1±25.3*	309.0±22.4	299.7±23.3**	298.1±24.9**(↓5%)
Week 52	424.9±65.2	406.2±51.2	415.9±49.9	401.9±61.2	386.6±54.2**(↓9%)
Week 102	446.9±92.7	427.5±92.1	497.0±74.0	461.9±82.8	427.3±77.3(↓4%)

*Significantly different from control p <0.05. **Significantly different from control p <0.01.
Data excerpted from pages 109-129 of the report.

Table 5. Body weight gain (g)					
Parameter	**0 ppm**	**20 ppm**	**200 ppm**	**2000 ppm**	**20000 ppm**
Males:					
Body weight gain, Week -2-13 (% control)	385.7	373.8 (97)	387.2 (100)	366.1 (95)	352.8** (91)
Body weight gain, Week -2-52 (% control)	575.1	591.1 (103)	577.7 (100)	567.6 (99)	527.6** (92)
Overall body weight gain (% control)	595.1	600.9 (101)	571.5 (96)	571.1 (96)	549.0 (92)
Females:					
Body weight gain, Week -2-13 (% control)	151.3	140.2* (93)	145.2 (96)	135.8** (90)	134.2** (89)
Body weight gain, Week -2-52 (% control)	261.0	242.4 (93)	252.1 (97)	238.0 (91)	222.6** (85)
Overall body weight gain (% control)	282.8	265.3 (94)	334.4 (118)	299.5 (106)	263.6 (93)

*Significantly different from control, p <0.05. **Significantly different from control, p <0.01.
Data excerpted from pages 151-162 of the report.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Reduction in food efficiency was noted over the first year at 20000 ppm (statistically significant) from 1-51 weeks. The overall (1-101 or 1-103 week) food efficiency was not significantly different from control. No effects were noted in any other dose group.

Statistically significant differences in food consumption or food efficiency were periodically observed in all dose groups during weekly/biweekly measurements. These changes were small, and the toxicological significance was doubtful.

Table 6. Food consumption (g) and food efficiency (%)

Parameter	0 ppm	20 ppm	200 ppm	2000 ppm	20000 ppm
Males:					
Food consumption Week 1-51 (% control)	26.96	25.90** (96)	26.58 (99)	26.13** (97)	27.21 (101)
Food consumption Week 1-103 (% control)	27.31	26.27 (96)	26.99 (99)	26.95 (99)	27.95 (102)
Food efficiency, Week 1-51 (% control)	4.82	5.15 (114)	4.84 (107)	4.79 (106)	4.35** (96)
Food efficiency, Week 1-103 (% control)	2.48	2.57 (104)	2.36 (95)	2.36 (95)	2.26 (91)
Females:					
Food consumption Week 1-51 (% control)	18.79	18.28 (97)	18.47 (98)	18.15** (97)	19.09 (102)
Food consumption Week 1-103 (% control)	19.01	18.67 (98)	19.14 (101)	18.55 (98)	19.61 (103)
Food efficiency, Week 1-51 (% control)	3.10	2.99 (96)	3.08 (99)	2.97 (96)	2.69** (87)
Food efficiency, Week 1-101 (% control)	1.69	1.59 (94)	2.14 (127)	1.89 (112)	1.60 (95)

* Significantly different from control, $p < 0.05$. ** Significantly different from control, $p < 0.01$.
Data excerpted from pages 172-176 & 183-188 of the report.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related ophthalmological lesions were observed at any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology and coagulation parameters

   There were sporadic changes in some of the parameters, and only one parameter (platelets) showed statistically significance at 6 month.. However, the magnitude of these changes was small; they were sporadic and lack of a dose response. Therefore, they are not treatment-related.

2. Clinical chemistry

At 6 month, no treatment-related changes in clinical chemistry parameters were observed. At the 12-month, increases in mean GGT (gamma glutamyltransferase), AST (aspartate

aminotransferase), ALT (alanine aminotransferase), and SDH (sorbitol dydrogenase) levels in 20000ppm males were observed (Table 7). However, the values for both the control and high dose group were highly variable. The increases were mainly seen in 3 male rats in the 20000 ppm group; two of these three males had associated liver histopathological changes (focal necrosis).(Table 9) At the end of the study, increase incidence of focal vacuolation was observed. The enzyme changes associated with focal necrosis of the liver were considered treatment-related and adverse.

Table 7. Clinical chemistry evaluation at 12 months

Parameter	0 ppm	20 ppm	200 ppm	2000 ppm	20000 ppm
Males: n=10					
ALT (Units/L)	63.9±81.8	34.4±8.0	35.6±6.5	29.4±5.8	135.4±197.0
AST (Units/L)	117.6±141.7	74.2±11.9	69.9±13.3	64.5± 16.0	175.9±248.2
GGT (Units/L)	1.6±0.7	1.3±0.5	1.3±0.5	1.6±0.5	3.3±2.8*
SDH (Units/L)	26.4±38.0	14.7±3.8	13.9±4.8	11.4±3.1	43.5±62.7

[a] Significantly different from control, $p < 0.05$. Data excerpted from pages 1258-1261 of the report.

3. Urinalysis

No test substance-related effects among urinalysis parameters were identified in either sex at any dietary concentration at any time point. There were occasional statistically significant changes that were not considered test substance-related due to their small magnitude, sporadic nature, and/or lack of a dose response.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Test substance-related increases in liver weight (variable statistical significance) were observed in males and females in the 2000 and 20000 ppm groups at the one-year interim sacrifice and in 20000 ppm males at the terminal sacrifice (Table 8). A few other statistically significant differences in organ weights were observed but were not considered related to test substance exposure as they lacked a dose-response and/or a microscopic pathology correlate.

Table 8. Absolute and relative Organ weights					
Parameter	**0 ppm**	**20 ppm**	**200 ppm**	**2000 ppm**	**20000 ppm**
Males:					
12 month					
Absolute liver weight (g)	19.1±2.6	18.5±1.7	18.5±3.1	22.1±3.6	20.0±2.8
Liver to body weight	2.6±0.3	2.6±0.2	2.6±0.2	3.0±0.2**	3.0±0.2**
Liver to brain weight	8.7±1.3	8.4±0.9	8.5±1.2	10.3±1.6*	9.3±1.4
24 month					
Absolute liver weight (g)	23.4±3.8	21.5±3.7	22.4±4.2	25.5±4.2	27.91±7.6*
Liver to body weight	3.2±0.6	2.8±0.3	3.1±0.4	3.5±0.6	4.1±1.4**
Liver to brain weight	10.5±1.7	9.7±1.8	10.1±1.8	11.5±1.9	12.6±3.5*
Females:					
12 month					
Absolute liver weight (g)	12.5±1.8	12.4±1.8	11.6±1.7	13.4±2.8	14.3±2.5
Liver to body weight	3.0±0.3	3.0±0.4	3.0±0.3	3.6±0.5**	3.8±0.6**
Liver to brain weight	6.3±1.0	6.3±0.9	6.0±0.9	6.9±1.7	7.2±1.0
24 month					
Absolute liver weight (g)	14.3±3.0	12.6±2.6	17.4±6.7	16.9±3.7	16.7±3.0
Liver to body weight	3.5±1.3	3.1±0.5	3.7±1.1	4.0±1.0	4.2±0.6
Liver to brain weight	7.1±1.4	6.3±1.4	8.7±3.5	8.5±2.0	8.5±1.6

*Significantly different from control, p <0.05. **Significantly different from control, p <0.01.
Data excerpted from pages 1701 -1742 of the report.

2. Gross pathology

   No test substance-related gross lesions were observed at necropsy at the interim or terminal sacrifice.

3. Histopathology

   Interim sacrifice (1-year)

   An increase in incidence of hepatocellular hypertrophy was seen in 2000 and 20000 ppm males and females. The hypertrophy was described as minimal centrilobular hypertrophy in males and minimal to mild centrilobular hypertrophy and panlobular hypertrophy (one animal) in females.

Terminal Sacrifice

Non-Neoplastic Findings

At terminal sacrifice, treatment-related histopathological changes in the liver were observed at 2000 and 20000 ppm males and females (Table 9). Centrilobular

hepatocellular hypertrophy of minimal severity was noted at 20000 ppm in both sexes and at 2000 ppm in females. This change is considered to reflect the proliferation of microsomal enzymes within the hepatocytes as a result of test substance administration and is not considered to be adverse. In males, increases in the incidences of foci of cellular alteration (clear, eosinophilic, and basophilic) and focal vacuolation were present at 2000 and 20000 ppm with variable statistical significance.

An increase in the incidence of chronic progressive nephropathy was seen in the kidneys of females at 2000 and 20000 ppm; at 20000 ppm the increase showed a statistical significance (Table 9). However, the incidences in these two dose level were similar (44/60 and 45/60 for 2000 and 20000 ppm, respectively) while the concentration difference was 10 times. Chronic progressive nephropathy is a common finding in aged rodents as reflected by the high incidence in the concurrent controls (34/60), and the current finding does not show a dose-related response. These two observations support the determination that the chronic progressive nephropathy seen in treated rats is not compound-related. At terminal sacrifice, there was an increase in incidence of erosion/ulcer and epithelial cell hyperplasia in non-glandular stomach of the 20000 ppm female relative to the controls (Table 9). These changes appeared to be treatment-related and occurred after prolong exposure (longer than 1 year).

Table 9. Incidences of non-neoplastic microscopic pathology

ppm	0	20	200	2000	20000
Males:					
12-month					
Liver: Centrilobular hypertrophy	0/10	0/10	0/10	5/10	8/10
Focal necrosis	0/10	0/10	2/10	1/10	2/10
24-month					
Liver: Centrilobular hypertrophy	0/60	0/60	0/60	0/60	6/60#*
Focus of cellular alteration, clear	0/60	0/60	1/60	4/60#	5/60#
Focus of cellular alteration, eosinophilic	20/60	15/60	12/60	29/60	32/60#*
Focus of cellular alteration, basophilic	10/60	9/60	9/60	12/60	20/60#
Vacuolation, focal	4/60	4/60	7/60	13/60#*	13/60#*
Females:					
12-month					
Liver: Centrilobular hypertrophy	0/10	0/10	0/10	4/10	6/10
Panlobular hypertrophy	0/10	0/10	0/10	0/10	1/10
Focal necrosis	0/10	0/10	0/10	1/10	1/10
24-month					
Liver: Centrilobular hypertrophy	0/60	0/60	0/60	9/60#*	22/60#*
Kidney: Nephropathy, chronic progressive	34/60	37/60	32/60	44/60	45/60#
Stomach, non-glandular: Erosion/ulcer	0/60	1/60	0/60	0/60	5/60
Epithelial hyperplasia	1/60	2/60	2/60	3/60	6/60

Significantly different from control by Cochran-Armitage trend test p <0.05.

* Significantly different from control by Fisher's exact test, p <0.05.

Note: Statistical analyses were not conducted on the microscopic findings at one year.

Data excerpted from pages 1744-1968 of the study report.

Neoplastic Findings

In males, there was an increase in the incidence of combined pancreas islet cell adenoma/carcinoma in 20 ppm (17%), 2000 ppm (22%), and 20000ppm (13%) relative to the controls (7%), and at 200 ppm the incidence was the same as the controls (Table 10). With Fisher's Exact Test, the increase of combined adenomas/carcinomas at 2000 ppm showed a statistical difference from the controls ($p<0.05$). At 20000 ppm, no statistical significance was demonstrated.

An increase in liver adenomas was seen in the 20000 ppm males but the increase did not demonstrate a statistical significance, and there was no dose-related response.

In females, the incidence of thyroid follicular cell adenomas was seen in all treatment groups and the controls (Table 10), and there was no dose-related response for adenomas. There was a slight increase in the incidence of thyroid follicular cell carcinomas at 2000 and 20000 ppm female, and the increase in 20000 ppm showed a significant trend with Cochran-Armitage trend test ($p=0.03$). Despite of a wide spread in the concentration levels between 2000 ppm and 20000ppm, the incidence of the thyroid follicular cell carcinomas were comparable between these two dos levels (2/59 vs 2/60). This historical provided by the registrant showed that the increase was within the range of the historical data for both thyroid follicular cell adenomas and carcinomas. In males, there was no increase in thyroid follicular cell adenomas or carcinomas at any dose level tested.

The pancreas islet cell tumor and liver tumor in males and thyroid follicular cell tumor in females were not treatment related for the following reasons: (1) the increase did not demonstrate a dose- related response, (2) no consistent statistical significant difference existed between the control and the treated groups, and (3) the increase was slight over a broad range of dosing concentrations. Specific to thyroid follicular cell tumor, male rats are known to be more sensitive to xenobiotic-induced proliferative lesions of thyroid follicular cells than the female rats (Capen, 1997); yet male rats in this study did not showed a treatment-related increase in thyroid follicular cell tumor. This further supports conclusion that thyroid follicular cell tumor seen in female rats is not be compound-related.

Table 10. Incidences of neoplastic microscopic pathology

Ppm	0	20	200	2000	20000
Male					
Pancreas, islet cell					
Adenomas	2/60	3/48	3/44	4/40	5/60
Carcinomas	2/60	5/48	0/44	5/40	3/60
adenoma/ carcinoma	4/60 (7%)	8/48 (17%)	3/44 (7%)	9/40# (22%)	8/60 (13%)
Liver					
Adenomas	1/60	2/60	1/60	2/60	4/60
Carcinomas	2/60	0/60	0/60	1/60	2/60
Adenomas/carcinomas	3/60	2/60	1/60	3/60	6/60
Female					
Thyroid gland					
Adenoma, follicular cell	1/60	3/60	1/60	1/59	2/60 (3.33%)
Carcinoma, follicular	0/60	0/60	0/60	2/59	2/60*(3.33%
Adenoma/ carcinoma	1/60	3/60	1/60	3/59	4/60 (6.67%)

Historical Control Range (MPI Research, Inc.) October 1999 to June 2010 (53 studies)[a]	
Thyroid	
Male: *Thyroid follicular cell adenomas (49 studies for males)*	*0 - 11.9%*
Thyroid follicular cell carcinomas	*0 - 2.9*
Female: *Thyroid follicular cell adenomas (53 studies for females)*	*0 - 5%*
Thyroid follicular cell carcinomas	*0 - 3.3%*
Pancreas	
Males: Islet cell adenomas (49 studies for males)	*0 - 18.3%*
Islet cell carcinomas	*0 - 7.1 %*
Females: Islet cell adenomas (53 studies for females)	*0 - 8.3%*
: Islet cell adenomas	*0 - 4.7%*

*: Statistically significant by the Cochran-Armitage trend test, p <0.05. # Statistically significant by the Fisher's exact test, p <0.05
Data excerpted from 2062-2088 of the report.
[a]: Historical control data excerpted from DuPont Project Identification : DuPont-34740; MRID 48734001. 02/13/2012

III. CONCLUSION

When groups of Crl:CD®(SD) rats were treated with cyantraniliprole for two year, the compound did not affect survival, clinical or ophthalmological observations, or gross pathology. Test substance-related effects were observed in liver after one year and in liver and kidney (females) after two years. After two years, the incidence of foci of cellular alteration (clear cell, eosinophilic and basophilic) and focal vacuolation was increased in livers of males at ≥2000 ppm (variable statistical significance). Considering all the data in this study, cyantraniliprole did not produce compound-related or dose-related increase in tumor incidence.

The no-observed-adverse-effect level (NOAEL) was 200 ppm (8.3 mg/kg bw/day), and LOAEL was 2000 ppm (84.8 mg/kg bw/day) based on microscopic liver pathology characterized by foci of cellular alteration (clear, eosinophilic, and basophilic) and focal vacuolation.

This study is classified as fully reliable (acceptable/guideline) and satisfied the guideline requirements (OPPTS 870.4300; OECD Section 4 (Part 453)) for a combined chronic oral toxicity/carcinogenicity study in rats.

Reference

Capen, CC (1997). Mechanistic Data and Risk Assessment of Selected Toxic End Point of the Thyroid Gland. Toxicologic Pathology 1997; 25:39-48.

Global Primary Reviewer: Whang Phang, PhD

IIA 5.5.3/01 Carcinogenicity study in the mouse

Report: **IIA** 5.5.3/01C raig, L. (2011); Cyantraniliprole (DPX-HGW86) technical: Oncogenicity study 18-month feeding study in mice. MPI Research, Inc., Mattawan, Michigan, USA. Laboratory Report No.: 125-100. DuPont-26843. April 26, 2011. MRID 48122578. Unpublished.

Guidelines: OECD Section 4 (Part 451) (1981 and 2009)
OPPTS 870.4200 (1998)
EEC Method B.32, Directive 88/302/EC (1988)
MAFF 12 Nousan 8147 (2000)

GLP: Yes OECD Principles of GLP,ENV/MC/CHEM (1998)
Singed and date GLP, Quality Assurance, and Data Confidentiality statements were presented in the report.

Executive summary:

In an 18-month carcinogenicity study (MRID 48122578), groups of male and female Crl:CD1®(ICR) mice (60 mice/sex/concentration) were fed cyantraniliprole (97.0%; HGW86-412) at dietary concentrations of 0, 20, 150, 1000, and 7000 ppm (males: 0, 2, 16, 104, and 769 mg/kg bw/day; females: 0, 2, 19, 131, and 904 mg/kg bw/day) for 18 months. Parameters evaluated included the following: body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, ophthalmology, organ weights, and gross and histopathology,

Under the conditions of this study cyantraniliprole did not produce treatment-related effects on survival, clinical or ophthalmological signs of toxicity, incidence of masses, or changes in clinical pathology parameters. Test substance–related increases in mean liver weight parameters were observed in males and females fed 1000 or 7000 ppm and correlated with increased incidence of hepatocellular hypertrophy at the same concentrations. In the 28 day oral toxicity study in mice, increased hepatic cytochrome P-450 contents were seen at comparable dose levels. Therefore, these changes appeared to be adaptive and not adverse.

Cyantraniliprole at 7000 ppm induced a statistically significant increase in food consumption and a decrease in food efficiency with very slight but little changes in body weights. These changes suggest that the 7000 ppm animals were unable to adequately utilize food, and it was a compound-related effect and was considered to be adverse. Therefore, the NOAEL was 1000 ppm (104 and 131 mg/kg bw/day for males and females, respectively). LOAEL was 7000 ppm (769 and 904 mg/kg bw/day for males and females respectively) based on the effects on food utilization.

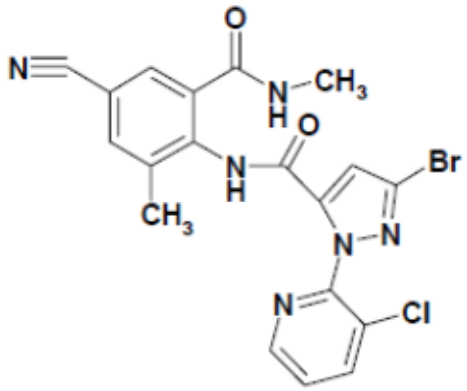
The highest dose 7000 ppm in this study was approaching the limit dose (1000 mg/kg bw/day) and did not produced compound-related increase in tumor incidence while producing effects on food utilization. .

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.4200; OECD Section 4 (Part 451)) for a carcinogenicity study in mice.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   3-bromo-N-[-4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide.



Lot/Batch #:	HGW86-412
Purity:	97.0%
Description:	White powder
CAS #:	736994-63-1
Stability of test compound:	Analyses confirmed that test substance was generally distributed uniformly in the feed and was present in the feed at targeted concentrations. Results from a 2-year rat feeding study (DuPont-26842) confirmed that the test substance was stable in feed for at least 14 days at room temperature.

2. Vehicle and/or positive control: Untreated diet
3. Test animals

Species:	Mouse
Strain:	Crl:CD-1®(ICR)
Age at dosing:	Approximately 57 days old
Weight at dosing:	20.1–39.3 gm for males; 19.9–27.9 gm or females
Source:	Charles River Laboratories, Inc., Portage, MI
Acclimation period:	15 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed

of all animals except negative controls.

Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly (males) or three per cage (females) in poly shoebox cages with non-aromatic bedding. If an animal died, the cage-mate was housed individually. Animals were provided with Nylabones as environmental enrichment.

4. Environmental conditions

Temperature:	22 ± 4°C
Humidity:	50 ± 20%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   11 December 2008 to 16 June 2010

2. Animal assignment and treatment

   Five groups of 60 animals/sex/concentration were administered cyantraniliprole in feed daily for 18 months, at dietary concentrations of 0, 20, 150, 1000, and 7000 ppm. Dietary concentrations were based on results of a 90-day study where no adverse effects were observed on clinical signs, clinical chemistry, survival, histopathology exposed to dietary concentrations up to 7000 ppm, (MRID 48119943). However, there was an increase in liver weights and increased incidence of liver hypertrophy at ≥1000 ppm females and 7000 ppm males; the effects were considered as adaptive response. However, there was an increase in the incidence of focal necrosis of the liver in the 7000 ppm females (4/10) and males (1/10). Focus of cellular alteration was also seen in one 7000 ppm male.

   Animals were assigned to dose groups by block randomisation so that there were no statistically significant differences among group body weight means within a sex (Table 1). A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table 1. Study Design

Group no.	No./ sex/group	Conc. in diet (ppm)[a]
1	60	0 (control)
2	60	20
3	60	150
4	60	1000
5	60	7000

a: weight/weight concentration of the test substance, adjusted for purity.

3. Diet preparation and analysis

The test substance was added to the rodent diet and thoroughly mixed for 20 minutes during weeks 1-5, then for 10 minutes for the remainder of the study. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The homogeneity and concentration of cyantraniliprole in the dietary mixtures were evaluated at the beginning of the study by analysis using HPLC/UV. Analysis for concentration verification was also conducted approximately every 3 months. Stability of the test substance in diet was evaluated as part of an accompanying 2-year study in rats (DuPont-26842, see point IIA 5.5.1 above); stability was verified for up to 14 days at room temperature.

The analytical results showed that the test substance in the test diet ranging from 83.2% to 101.8% of the nominal concentrations. The beginning of the study, homogeneity test results demonstrated that the diet was well mixed (1.8-4.0% RSD). Throughout the study, diets were at targeted concentrations (81.3-109% of nominal concentrations) except for the 20 ppm diet, which was not within $\pm$20% of nominal concentration at two evaluations. Analysis of diets from a rat feeding study (DuPont-26842) verified that the test substance was stable for up to 14 days at room temperature.

4. Statistics:

The statistical methods employed for analyzing the results of this study are summarized in Table 2. The methods used were appropriate.

Table 2. Statisticsal methods applied on the 18-month feeding study in mice

Parameter	
Body weight, body weight gain, food consumption, organ weight, haematology, clinical chemistry, coagulation	Group Pair-wise comparisons: Levene's test for homogeneity. If not significant, then Dunnett's test. If Levene's test was significant, then Welch's t-test with Bonferroni correction.
Food efficiency	Rank transformation with Dunnett's test
Mortality data	Kaplan-Meier product-limit method
Microscopic pathology data (neoplastic and non-neoplastic)	Cochran-Armitage trend test Fisher's exact test

Note: Significance was judged at $p < 0.05$ and < 0.01. Separate analyses were performed on the data collected for each sex.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and morbidity and examined for detailed clinical signs of toxicity weekly for the first 13 weeks then every other week thereafter.

2. Body weights

   All animals were weighed once per week. No body weight was recorded on the first day of dosing. Therefore, all body weight gains were calculated from the week -1 (day -1) starting value.

3. Food consumption, food efficiency and daily intake

   Food consumption was recorded for each animal weekly for the first 13 weeks then every other week thereafter. Food efficiency and daily intake (compound consumption) were calculated from food consumption and body weight data.

4. Ophthalmological examinations

   All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (hematology)

   Blood samples were collected from all animals approximately 12 and 18 months after initiation of the study and from animals sacrificed *in extremis*. Animals sacrificed *in extremis* were evaluated for erythrocyte count and leukocyte count (total and absolute differential). Animals in the control and high dose (7000 ppm) groups at terminal sacrifice were evaluated for differential leukocyte count.

6. Sacrifice and pathology

   At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all animals (terminal sacrifice and early deaths). Organs that were collected and weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (7000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically. All tissues from animals that died prior to scheduled sacrifice were evaluated. In addition the following tissues were evaluated in the 20, 150 and 1000 ppm groups: both sexes - liver, eyes, heart, and stomach; males only - kidneys, thyroid gland, urinary bladder; females only - femur with bone marrow, mesenteric lymph nodes, and ovaries. Subsequent histopathology of these tissues revealed that only the liver had test substance-related microscopic findings.

Table 3. Organs/tissues collected for pathological examination

Tissue	Organ weight taken	Collected and preserved	Microscopic examination	
			1, 5[a]	2-4[a]
Adrenal gland	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	

Tissue	Organ weight taken	Collected and preserved	Microscopic examination	
			1, 5[a]	2-4[a]
Epididymis	X	X	X	
Esophagus		X	X	
Eye (with optic nerve and retina)		X	X	
Gallbladder		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidney[a]	X	X	X	
Lacrimal gland, exorbital		X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	X
Lung with bronchi		X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (process females only)		X	X	
Nerve, sciatic		X	X	
Nose		X	X	
Ovary	X	X	X	
Oviducts		X	X	
Pancreas		X	X	
Peyer's patch		X	X	
Pharynx		X	X	
Pituitary		X	X	
Prostate		X	X	
Salivary gland, mandibular		X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Seminal vesicles		X	X	
Skeletal muscle, biceps femoris		X	X	
Skin		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, glandular		X	X	
Stomach, non-glandular		X	X	
Target Organs		X	X	X
Testis	X	X	X	
Thymus		X	X	
Thyroid gland (with parathyroid)[a]	X	X	X	
Tongue		X	X	
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	

Tissue	Organ weight taken	Collected and preserved	Microscopic examination	
			1, 5[a]	2-4[a]
Uterus with cervix	X	X	X	
Vagina		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node		X	X	X

[a]: These numbers refer to dose groups as shown in Table 1.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs of toxicity were observed at any dietary concentration in either males or females.

2. Mortality

Study survival was similar in all groups, with the exception of 20 and 150 ppm males, whose survival was significantly greater than controls. There were many different causes of death in mice which died before the scheduled sacrifice, and all were considered typical of naturally occurring incidental or age-related disease. None were attributed to exposure to the test substance.

Table 4. Survival rates at the end of the study (week 79)

ppm	Males	Females
0	47/60	50/60
20	57/60	51/60
150	56/60	50/60
1000	49/60	49/60
7000	50/60	44/60

Data excerpted from pages 45-48 of the report.

BODY WEIGHT AND BODY WEIGHT GAIN

The mean body weights of the 20, 150, and 1000 ppm males were comparable to those of the controls, but the body weight for 7000 ppm males was decreased relative to the controls (Table 5 and Figure 1). At times the body weight decrease in 7000 ppm showed a statistical significance. The body weights of treated females were comparable to those of the controls.(Table 5 and Figure 2).

Table 5. Body Weights at Selected Study Intervals (gm)					
Week in Study	0 ppm	20 ppm	150 ppm	1000 ppm	7000 ppm
	Males				
-1	34.0±2.0	33.9±2.1	33.7±2.0	33.8±1.9	34.0±2.8
1	34.3±2.1	34.8±2.2	34.9±2.3	34.9±2.0	35.1±2.1
22	42.7±0.1	43.0±4.3	41.7±4.7	42.0±4.2	41.0±3.4
56	46.1±4.8	46.7±5.0	45±7.0	45.6±5.2	43.7±4.8
78	44.2±4.7	44.6±4.7	43.8±7.2	43.3±5.7	43.1±4.8
	Females				
-1	23.7±1.5	23.4±1.6	23.5±1.4	23.9±1.5	24.0±1.5
1	24.7±1.7	24.7±1.7	23.7±1.4	23.9±1.5	25.1±1.6
22	35.0±3.7	34.3±4.1	34.0±3.9	33.1±3.1	33.4±3.4
56	39.3±4.5	40.0±6.6	38.4±6.3	37.3±4.8	37.9±4.4
78	38.0±4.1	41.2±8.7	38.4±6.9	36.2±4.9	37.3±5.5

Data excerpted from pages 68-83 of the report.

Figure 1. Male - Mean Body Weights

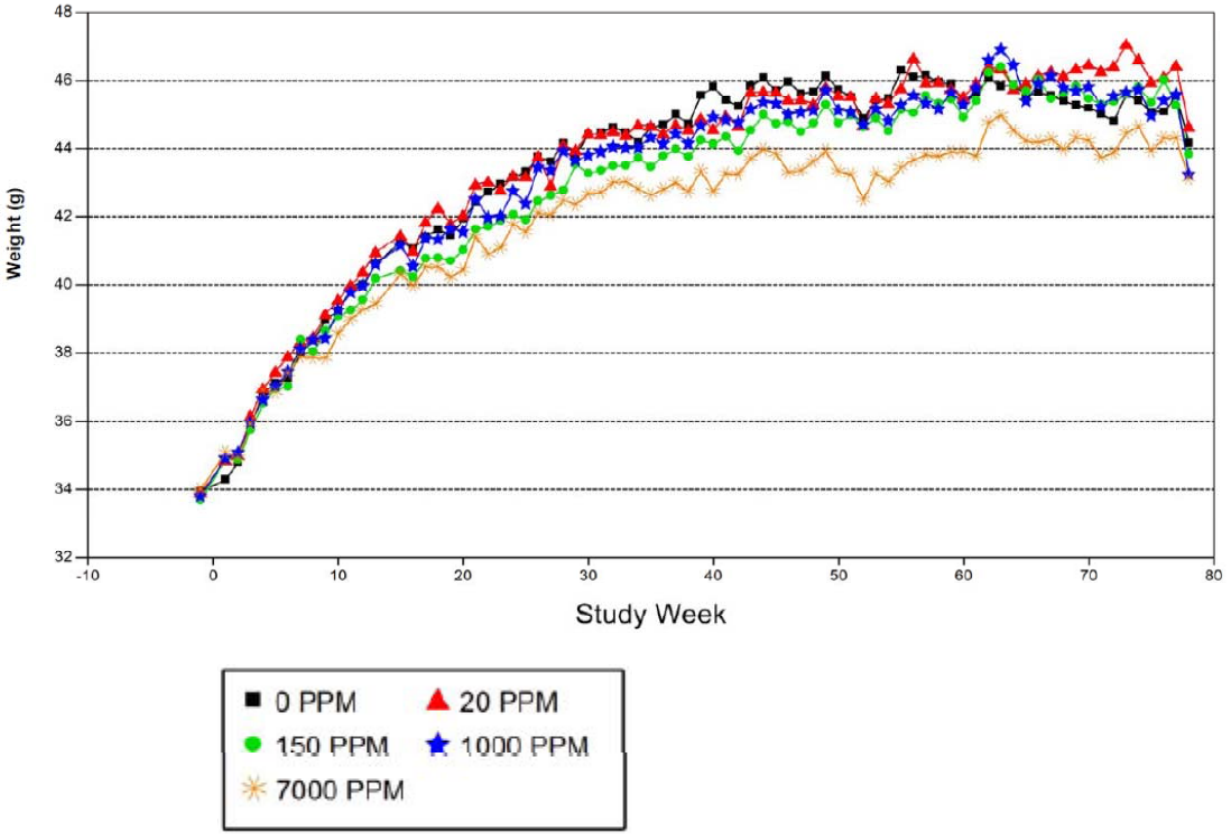


Figure excerpted from page 39 of the study report.

Figure 2. Female - Mean Body Weights

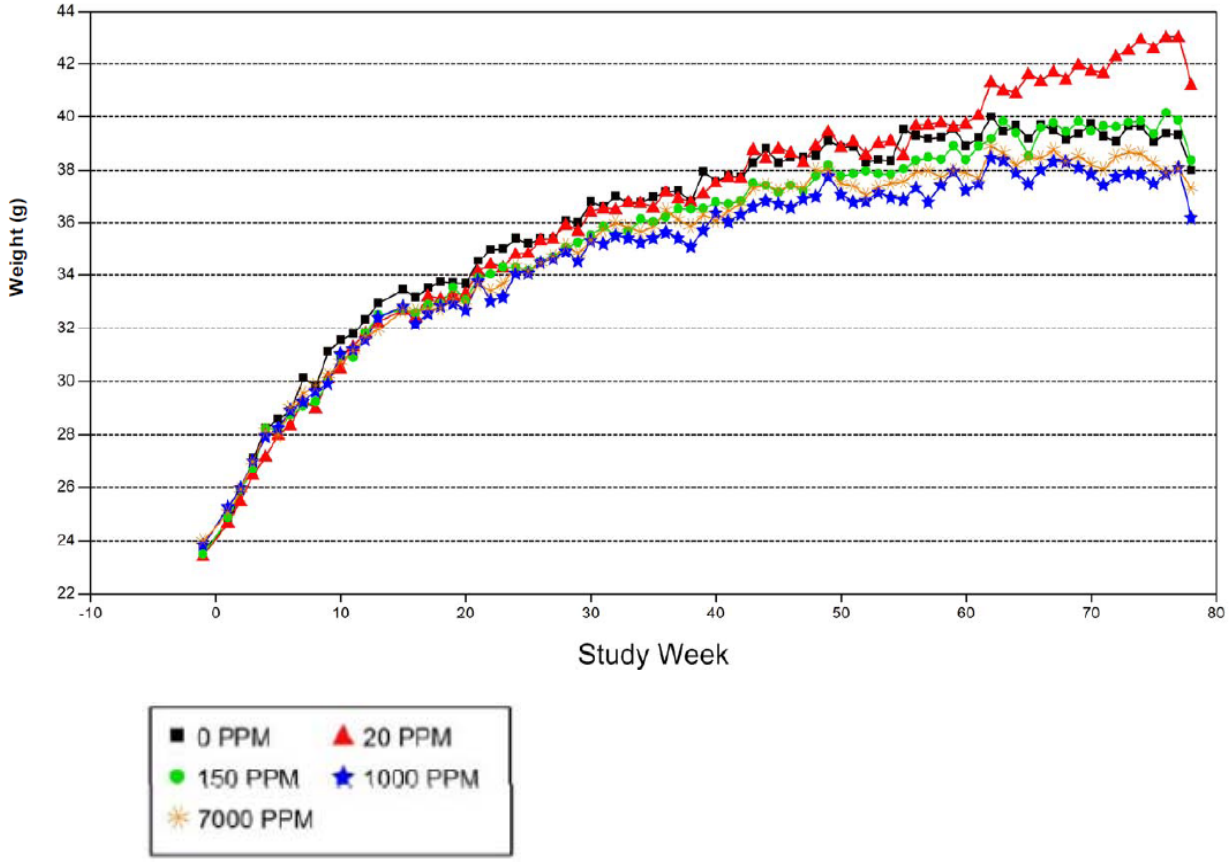


Figure excerpted from page 40 of the report.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

Mean food consumption in the male 7000 ppm and female 1000 and 7000 ppm groups was greater than control values over much of the study, including the weeks 1-52 and 1-77 intervals (Table 6). The increase in food consumption also showed statistical significance. Food efficiency was deceased at 7000 ppm males and females; the decrease was statistically significant. The increase in food consumption with associated decreased in food efficiency and very little changes in body indicated the test animals were unable to use utilize food efficiently. This set of data suggests a compound-related effect and it might be adverse. in these groups over these intervals. The main daily compound consumption is presented in Table 7.

Parameters	Table 6. Food Consumption					
	Interval (Weeks)	**0 ppm**	**20 ppm**	**150 ppm**	**1000 ppm**	**7000 ppm**
		Males				
Food Consumtion gm/animal/day)	1-13	4.8 ±0.5	4.8±0.4	4.7±0.5	4.8±0.4	5.0±0.5
	1-52	4.6±0.3	4.7±0.4	4.6±0.4	4.7±0.4	4.9±0.4**
	1-77	4.6±0.3	4.6±0.4	4.6±0.4	4.7±0.4	4.8±0.4**
Food Efficiency (%)	1-13	1.47	1.42	1.27	1.30	0.98**
	1-51	0.67	0.63	0.60	0.59	0.47**
	1-77	0.45	0.46	0.42	0.42	0.36**
Females						
Food consumption (gm/animal/day)	1-13	4.4±0.5	4.3±0.3	4.3±0.5	4.7±0.7	4.4±0.3
	1-52	4.2±0.4	4.3±0.3	4.3±0.4	4.5±0.7**	4.4±0.2**
	1-77	4.1±0.3	4.3±0.2	4.2±0.3	4.4±0.5	4.3±0.2*
Food Efficiency (%)	1-13	2.31	2.33	2.24	2.08	2.00
	1-51	1.02	0.98	0.90	0.79**	0.83**
	1-77	0.71	0.84	0.69	0.59*	0.59*

*Statistically significant ($p<0.5$) **Statistically significant ($p<0.01$)
Data excerpted from pages 122-130 & 144-155 of the report.

Table 7. Mean Daily Compound Intake (mg/kg/day)		
Dietary Concentrations (ppm)	Males	Females
0 (control)	0	0
20	2	2
150	16	19
1000	104	131
7000	769	904

Data excerpted from pages 157-162 of the report. The values were rounded to the whole number.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related changes in the incidences of ophthalmological observations were observed at any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Hematology

Treatment-related effects on erythrocyte count and leukocyte count (total and absolute differential) were not found.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

A slight increase in liver weight was observed in males and females at ≥1000 ppm (with variable statistical significance) (Table 8). A few other organ weights were found to be different from the controls with variable statistical significance, but they were not treatment-related for they failed to show a dose-response and/or an associated histopathological change.

Table 8. Liver Weights

Parameter	0 ppm	20 ppm	150ppm	1000 ppm	7000 ppm
Males:					
Absolute liver weight (g)	2.1±0.6	2.1±0.4	2.1±0.4	2.4±0.6	2.4±0.5[a]
Liver to body weight (%)	4.9±1.5	4.8±0.7	4.9±0.7	5.5±1.5	5.6±1.2*
Liver to brain weight (ratio)	4.3±1.2	4.2±0.8	4.3±0.8	4.8±1.2*	4.8±1.2
Females:					
Absolute liver weight (g)	1.8±0.8	1.8±0.4	1.8±0.3	1.9±0.3	2.2±0.6**
Liver to body weight (%)	5.0±2.2	4.6±1.0	4.8±0.8	5.5±0.8	5.9±1.2**
Liver to brain weight (ratio)	3.6±1.8	3.7±0.8	3.6±0.6	3.9±0.6	4.4±1.2**

* Significantly different from control, $p < 0.05$. ** Significantly different from control, $p < 0.01$.
[a]: Excluding an outlier which had polycystic liver at 7000 ppm.
Data excerpted from pages 1039-1057 of the report.

2. Gross pathology and histopathology

No treatment-related gross findings were observed. Histopathology data demonstrated that there was a statistically significant increase in the incidence of liver centrilobular hypertrophy in 1000 and 7000 ppm males and females (Table 9). The hypertrophy was graded as minimal to mild and was associated with increase in liver weights; additional dose-related increase in liver lesions was not found. The cyantraniliprole induced liver effects appear to be an adaptive effect as supported by the data from the 28-day study where increases in liver weights, incidence of liver hypertrophy were accompanied by increases in hepatic cytochrome P45 contents. Table 9 presents representative non-neoplastic histological findings in selective organs. Hence, with the exception of liver hypertrophy, other dose-related increase in the incidence of non-neoplastic findings was not seen.

Tables 10 and 11, present the selective summary data on primary tumor incidence. There was an increase in systemic histiocytic sarcoma in 7000 ppm females (4/60) and one female at 60 ppm also had this tumor (Table 10); similar increase was not found in males. The histiocyte sarcoma seen in 7000 ppm females was not compound-related for three reasons. (1) The increase in this tumor incidence was marginal, (2) the dose, 7000 ppm (904 mg/kg bw/day), was approaching the limit dose (1000 mg/kg bw/day) for carcinogenicity study, and (3) no other effects on any of associated haematological parameters were observed. A tumor summary for all tumor types is presented in Table 12; the tumor incidence in treated and control mice was comparable. With these considerations it was concluded that, under the conditions of this study, no compound-related increase in tumor incidence was observed in mice.

Table 9. Incidences of non-neoplastic microscopic pathology on selective organs some of which were shown to be affected by cyantraniliprole in short term studies in mice

	0 ppm	20 ppm	150 ppm	1000 ppm	7000 ppm
Males:					
Liver: Centrilobular hypertrophy	3/60	1/60	0/60	**13/60***	**36/60***
Necrosis, focal / multifocal	2/60	2/60	1/60	2/60	3/60
Fatty change diffuse	3/60	0/60	6/60	1/60	0/60
Thyroid: Folicullar cysts	3/59	1/8	0/9	1/9	1/55
Kidney: Dilated tubules, focal/multifocal	31/60	31/60	27/60	16/60	33/60
Adrenal: Microvesiculation diffuse	6/59	0/9	0/6	0/10	6/60
Epididymides : Inflammation	1/60	0/4	0/5	0/10	1/60
Spermatocele	3/60	0/4	0/5	0/10	0/60
Lungs: Fibosis	1/60	0/11	0/4	0/18	2/60
Brain: Malacia, focal	2/60	0/3	0/4	0/10	4/60
Females:					
Liver : Centrilobular hypertrophy	0/59	0/59	0/59	**5/60***	**9/60***
Necrosis, focal / multifocal	2/59	6/59	3/59	4/60	7/60
Fatty change diffuse	0/59	2/59	0/59	0/60	0/60
Thyroid: Folicullar cysts	3/60	1/8	0/9	0/9	1/55
Kidney: Dilated tubules, focal/multifocal	21/60	6/11	5/12	6/11	21/60
Adrenal: Microvesiculation diffuse	0/59	1/9	0/9	0/9	0/58
Ovary: Atrophy	7/58	3/59	4/58	2/59	5/58
Hemorrhage	4/58	5/59	4/58	6/59	4/58
Hyperplasia	1/58	2/59	2/58	3/59	0/58
Lungs: Fibosis	0/60	0/12	0/14	1/17	0/60
Brain: Malacia, focal	3/60	0/9	1/10	0/11	3/59

*Significantly different from control by Cochran-Armitage trend test $p < 0.05$.
Data excerpted from pages 1305-1320 (males) and 1321-1340 (females) of the study report.

Table 10. Incidence of Primary Tumors in Male Mice

Number of Animals on Study :	0 ppm 60	20 ppm 60	150 ppm 60	1000 ppm 60	7000 ppm 60
ADRENAL GLANDS;					
Examined	(59)	(9)	(6)	(10)	(60)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	1	0	0	0	0
*b: subcapsular cell adenoma, unilateral	1	1	0	0	0
KIDNEYS;					
Examined	(60)	(60)	(60)	(60)	(60)
*b: renal tubular adenoma	1	0	0	2	0
LIVER;					
Examined	(60)	(60)	(60)	(60)	(60)
*b: hepatocellular adenoma	3	2	5	3	4
*b: hepatocellular adenoma, multiple	1	2	0	2	0
*m: hemangiosarcoma	1	1	2	1	0
*m: hepatocellular carcinoma	2	0	3	3	0
LUNGS;					
Examined	(60)	(11)	(4)	(18)	(60)
*b: bronchiolo-alveolar adenoma	8	3	0	3	6
*b: bronchiolo-alveolar adenoma, multiple	1	0	0	0	2
*m: bronchiolo-alveolar carcinoma	4	2	0	3	6
SYSTEMIC NEOPLASMS;					
Examined	(60)	(3)	(6)	(12)	(60)
*m: granulocytic leukemia	0	0	0	1	0
*m: histiocytic sarcoma	0	0	2	1	1
*m: malignant lymphoma	3	0	2	1	0
TESTES;					
Examined	(60)	(5)	(4)	(13)	(60)
*b: leiomyoma	0	0	0	1	0
*b: leydig cell adenoma, unilateral	0	0	0	2	1
*m: leydig cell carcinoma, bilateral	0	0	0	0	1
THYROID GLAND;					
Examined	(58)	(60)	(59)	(60)	(60)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	2	0	1	0	0
*b: thyroid follicular cell adenoma	1	0	0	1	0

Data excerpted from pages 1396-1398 of the study report.

Table 11. Incidence of Primary Tumors in female mice

Number of Animals on Study :	0 ppm 60	20 ppm 60	150 ppm 60	1000 ppm 60	7000 ppm 60
ADRENAL GLANDS;					
Examined	(59)	(9)	(9)	(11)	(58)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	0	1	1	0	2
Not Examined: MISSING	1	0	0	0	0
*b: subcapsular cell adenoma, unilateral	1	0	0	0	0
LIVER;					
Examined	(59)	(59)	(59)	(60)	(60)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	1	1	1	0	0
*b: hemangioma	0	0	1	0	0
*b: hepatocellular adenoma	3	1	0	0	0
*m: hemangiosarcoma	1	0	0	0	2
*m: hepatocellular carcinoma	1	0	0	0	0
LUNGS;					
Examined	(60)	(12)	(14)	(17)	(60)
*b: bronchiolo-alveolar adenoma	5	1	3	4	7
*b: bronchiolo-alveolar adenoma, multiple	1	1	0	0	1
*m: bronchiolo-alveolar carcinoma	2	1	3	2	2
MAMMARY GLANDS;					
Examined	(60)	(7)	(11)	(10)	(58)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	0	0	0	0	1
Not Examined: MISSING	0	2	0	1	1
*m: mammary gland adenocarcinoma	0	0	1	0	0
OVARIES;					
Examined	(58)	(59)	(58)	(59)	(58)
Not Examined: AUTOLYSIS: NO MICROSCOPIC EXAMINATION	2	1	1	1	2
Not Examined: MISSING	0	0	1	0	0
*b: cystadenoma, ovarian	0	0	1	0	0
*b: sex-cord/stromal tumor, bilateral	0	0	0	0	1
*b: sex-cord/stromal tumor, unilateral	2	0	4	1	3
*m: choriocarcinoma	1	0	0	0	0
PITUITARY GLAND;					
Examined	(60)	(9)	(8)	(11)	(58)
Not Examined: MISSING	0	0	1	0	0
Not Examined: INSUFFICIENT TISSUE	0	0	1	0	2
*b: pituitary adenoma, pars distalis	3	0	0	0	0
SYSTEMIC NEOPLASMS;					
Examined	(60)	(18)	(11)	(14)	(60)
*m: granulocytic leukemia	1	0	0	0	1
*m: histiocytic sarcoma	0	1	0	0	4
*m: malignant lymphoma	7	9	1	5	7

Data excerpted form pages 1399-1401 of the study report.

Table 12. Tumor summary for male and female mice

Removal Reason: All of those selected	MALE				
	0 ppm	20 ppm	150 ppm	1000 ppm	7000 ppm
Animals Examined	(60)	(60)	(60)	(60)	(60)
Tumour Bearing Animals	24	11	16	22	20
Animals with Malignant Tumours	10	4	10	12	8
Animals with Benign Tumours	17	8	6	14	15
Animals with Multiple Tumours	7	2	5	8	6
Animals with Single Tumours	17	9	11	14	14
Animals with Multiple Malignant Tumours	1	0	0	0	0
Animals with Multiple Benign Tumours	1	1	0	2	1
Animals with Metastasising Tumours	3	0	4	3	2
Total Tumours	69	13	54	36	30
Total Malignant Tumours	11	4	10	12	8
Total Benign Tumours	18	9	6	16	16
Total Metastasising Tumours	3	0	4	3	2

Table 12 (continued). Tumor summary for male and female mice

Removal Reason: All of those selected	FEMALE				
	0 ppm	20 ppm	150 ppm	1000 ppm	7000 ppm
Animals Examined	(60)	(60)	(60)	(60)	(60)
Tumour Bearing Animals	24	16	15	13	27
Animals with Malignant Tumours	14	13	7	8	16
Animals with Benign Tumours	15	4	11	5	13
Animals with Multiple Tumours	15	10	6	5	17
Animals with Single Tumours	9	6	9	8	10
Animals with Multiple Malignant Tumours	1	1	0	0	2
Animals with Multiple Benign Tumours	3	0	0	0	3
Animals with Metastasising Tumours	9	10	2	5	13
Total Tumours	156	71	22	24	146
Total Malignant Tumours	15	14	7	8	18
Total Benign Tumours	18	4	11	5	16
Total Metastasising Tumours	9	10	2	5	13

Data excerpted from pages 1303 and 1404 of the study report.

III. CONCLUSION

Under the conditions of this study, cyantraniliprole at 7000 ppm induced a statistically significant increase in food consumption and a decrease in food efficiency with very little changes in body weights. These changes suggest that the test animals were unable to adequately utilize food, and it was a compound-related effect and was considered to be adverse. Therefore, the NOAEL was 1000 ppm (104 and 131 mg/kg bw/day for males and females, respectively). LOAEL was 769 and 904 mg/kg bw/day for males and females respectively). The highest dose 7000 ppm in this study was approaching the limit dose (1000 mg/kg bw) and did not produced compound- related increase in tumor incidence.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.4200; OECD Section 4 (Part 451)) for a carcinogenicity study in mice.

Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIA 5.8/12

Report: Wagner, V.O., Jois, M. (2010); IN-PLT97: Bacterial reverse mutation assay. BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.: AD08NK.503.BTL. DuPont Report No.: DuPont-30552, Revision No.1. Study completion date: October 08, 2010, Amended Final Report Date: October 20, 2010. MRID 48122580. Unpublished.

Guidelines: OPPTS 870.5100
EU 2000/32/EC
OECD 471 (1998)
JMAFF 12-Nousan-8147 (2000)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In a bacterial reverse mutation assay (MRID 48122580), IN-PLT97 (98.1% purity) was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). In the toxicity-mutation phase (Trial I), nominal dose levels of 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate were tested. Based on the toxicity-mutation test, the dose levels of 0, 50, 150, 500, 1500, and 5000 μg/plate were tested in the confirmatory mutagenicity test (Trial II). The test substance was administered to the test system as a workable suspension in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL.

Test substance precipitation was observed starting at 1500 μg/plate for all tester strains both in the non-activation and activated testing systems in both Trials I and II. In the toxicity-mutation test, no appreciable toxicity was observed. In the confirmatory mutation test (Trial II), the number of revertants at all concentrations of the test substance was similar to concurrent controls in both with and without S9-activation test systems.

Under the conditions of this study, IN-PLT97 was negative for mutagenic activity in non-activated and S9-activated test systems.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for a **b**acterial reverse mutation assay (OPPTS 870.5100; OECD 471).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-PLT97 technical metabolite
   Lot/Batch #: PLT97-003
   Purity: 98.1%
   Description: Off-white powder
   CAS # Not available
   Stability of test compound: Data from the analysis of the samples for the study indicate that the test substance was at the targeted concentrations and stable when stored at room temperature during the dosing period in the vehicle.
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)

Positive control, non activation:

Positive control	Dose	Strain(s)
2-Nitrofluorene	1.0 µg/plate	TA98
Sodium azide	1.0 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
Methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-Aminoanthracene	1.0 µg/plate 2.0 µg/plate 10 µg/plate	TA98, TA1535, TA1537 TA100 WP2 *uvr*A

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2581, 2626, 2639
   Source: Moltox, Boone, NC
   Protein content: 37.5, 39.9, or 34.0 mg/mL
   Characterisation: Each bulk preparation of S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to Salmonella typhimurium TA100.

   S9 mix composition
   Sodium phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM
   $MgCl_{2:}$ 8 mM

4. Test organisms

   Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

   Trial 1: IN-PLT97 concentrations of 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate were evaluated in duplicate in the presence and absence of S9 activation.

   Trial 2: IN-PLT97 concentrations of 50, 150, 500, 1500, and 5000 μg/plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   June 29, 2010 to September 03, 2010

2. Plate incorporation assay

   This study consisted of 2 independent trials: a preliminary toxicity-mutation phase (Trial I) and a mutagenicity phase (Trial II). In both trials, 0.5 mL of S9 or Sham mix, 100 μL of tester strain (cells seeded) and 50 μL of vehicle or test substance dilution were added to 2.0 mL of molten selective top agar at 45 ± 2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 μL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37 ± 2°C. Plates that were not counted immediately following the incubation period were stored at 2 to 8°C until colony counting could be conducted. All toxicity-mutation test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in duplicate. All mutagenicity test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in triplicate.

   Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Toxicity was scored relative to the concurrent negative control plates and recorded with the mean revertant count for the strain, condition and concentration. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity.

3. Statistics

   Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the

presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

A test substance was classified as positive when the mean number of revertants in any strain except TA1535 and TA1537 and at any test substance concentration was at least 2 times greater than the mean number of revertants in the concurrent negative control and occurred in a positive dose-response relationship. For strains TA1535 and TA1537 a mean number of revertants of at least 3 times greater than negative control was needed to be considered a positive response.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Dosing formulations were analyzed by the Sponsor. No test substance was detected in the vehicle control samples. The analytical results indicate that the regulatory-required top dose level was achieved in the confirmatory mutagenicity assays and the results support the validity of the study conclusion. The stability analysis of the formulations found the test substance to be stable in DMSO at room temperature for the period of dosing.

Dosing formulations were adjusted for the initial Sponsor-reported purity (96.7%), using a correction factor of 1.034. However, the Certificate of Analysis reports a purity of 98.1% active ingredient. Nominal values based on the initially reported purity are consequently 101.4% of the intended dose levels based on the purity stated on the Certificate of Analysis.

B. MUTATION ASSAYS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance formed a workable suspension in DMSO at a maximum concentration of approximately 100 mg/mL. Due to an unacceptable dose formulation analysis result for the Experiment B2, the confirmatory mutagenicity assay was repeated and named, Experiment B3; due to an unacceptable positive control value in the retest, tester strain TA1537 in the presence of S9 activation was not evaluated but was retested again in Experiment B4. No positive mutagenic responses were observed at any dose level or with any tester strain in either the absence of presence of S9 metabolic activation in Trials I or II. The results from Experiment B1 and B3 are excerpted from the report and presented in Tables 1 and 2. No appreciable toxicity was observed. Test substance precipitation was observed starting at 1500 μg/plate for all tester strains both in the non-activation and activated testing systems in both Trial I and Trial II.

Table 1. Summary of average revertants/plate without activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I[a]	Trial II[b]	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-PLT97	0	32	15	94	108	18	17	11	5	37	34
	1.5	32	ne	121	ne	18	ne	6	ne	37	ne
	5.0	37		95		25		7		32	
	15	41		101		15		8		27	
	50	43	15	118	115	10	15	5	8	29	36
	150	36	16	104	116	23	19	8	13	31	32
	500	40	12	111	124	27	20	5	7	34	36
	1500	33	10	98	121	22	20	8	9	44	42
	5000	40	8	101	142	20	19	6	8	39	34
Positive control	-	168	256	678	796	530	650	489	824	468	428

[a] Average of 2 replicates.
[b] Average of 3 replicates (results for original test and retest).
[C]: 2-Aminoanthracene ne = Not evaluated
Data excerpted from pages 21-22 (Trail I; Experiment B1) and 29-30 (Taril II; Experiment B3)

Table 2. Summary of average revertants/plate with activation

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I[a]	Trial II[b]	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
IN-PLT97	0	39	20	102	127	11	11	4	11	46	44
	1.5	37	ne	119	ne	14	ne	3	ne	51	ne
	5.0	30		96		9		4		43	
	15	39		118		13		6		38	
	50	50	23	97	109	9	16	8	10	46	36
	150	43	17	122	132	14	10	9	6	44	41
	500	40	22	114	138	16	16	6	8	46	48
	1500	35	13	114	142	10	19	3	7	59	36
	5000	39	11	107	146	13	14	4	7	37	49
Positive Control[c]	-	465	344	803	769	69	92	60	89	247	181

[a] Average of 2 replicates.
[b] Average of 3 replicates (results for original test and retest).
[C]: 2-Aminoanthracene ne = Not evaluated
Data excerpted from pages 23-24 (Trail I; Experiment B1) and 31-32 (Trail II; Experiment B3) of the study report.

III. CONCLUSION

IN-PLT97 was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIA 5.8/08

Report: Madraymootoo, W., Jois, M. (2011); IN-PLT97: *In vitro* mammalian chromosome aberration test. BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.: AD08NK.341.BTL. DuPont Report No.: DuPont-30551, Revision No. 1. MRID 48122581. Study completion date: October 29, 2010. Revised final date: April 12, 2011. Unpublished.

Guidelines: OPPTS 870-5375
EC 2000/32/EC, No. L136 Annex 4A-B10
OECD 473 (1998)
JMAFF 2000
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In an *in vitro* mammalian chromosome aberration assay (MRID 48122581), IN-PLT97 (98.1% purity) was tested using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Doses were 50, 100, 200, 400, 800, and 1550 μg/mL for the non-activated and S9-activated 4-hour exposure groups, and 25, 50, 100, 200, 500, 1000, and 1550 μg/mL for the non-activated 20-hour exposure group. The highest dose level was set based on the production of cytotoxicity in a preliminary toxicity assay. Evaluations were necessary only at 100 μg/mL and above in the non-activated 4-hour and 20-hour exposure groups, and at 50 μg/mL and above in the S9-activated 4-hour exposure group. The test substance was administered to the test system as a suspension in dimethyl sulfoxide (DMSO). HPBL were treated for 4 hours (activated test system), and 4 and 20 hours (non-activated test system). After exposure to Colcemid®, metaphase cells were harvested 20 hours following the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition) then structural and numerical chromosome aberrations.

No statistically significant increases in structural chromosome aberrations were observed at any of the concentrations evaluated. Positive controls induced the appropriate response. In the non-activated 4-hour exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations, 1550 μg/mL, mitotic inhibition was 24%, relative to the solvent control. In the S9-activated 4-hour exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations, 500 μg/mL, mitotic inhibition was 63%, relative to the solvent control. In the non-activated 20-hour exposure group, at the highest test concentration evaluated microscopically for chromosome aberrations, 1550 μg/mL, mitotic inhibition was 25%, relative to the solvent control.

Based on the findings of this study, IN-PLT97 was concluded to be negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes with and without an exogenous metabolic activation system.

This study is fully reliable (acceptable/guideline) and satisfies the requirements for an *in-vitro* mammalian chromosome aberration assay (OPPTS 870-5375; OECD 473).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-PLT97 technical metabolite
   Lot/Batch #: PLT97-003
   Purity: 98.1%
   Description: Off-white powder
   CAS # Not available
   Stability of test compound: Results from analysis of the dosing solutions indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions and was stable under the conditions of the study.
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)
   Positive, non-activation: Mitomycin C (MMC) in water at 0.3 and 0.6 µg/mL
   Positive, activation: Cyclophosphamide (CP) in water at 10 and 15 µg/mL
3. Activation: Rat liver S9 (Aroclor 1254-induced)
   Lot number: 2521
   Source: Moltox, Boone, NC
   Protein content: 40.9 mg/mL
   Characterisation: Each bulk preparation of S9 was assayed for sterility and its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition
   Glucose-6-phosphate: 1 mM
   NADP: 1 mM
   KCl: 6 mM
   $MgCl_2$: 2 mM
   S9: 20 µL per mL medium

4. Test cells

   Human lymphocytes obtained from human venous blood from normal, healthy donors.

5. Culture medium

RPMI 1640, supplemented with 100 units penicillin/mL and 100 µg streptomycin/mL, and 2 mM L-glutamine.

6. Test compound concentrations evaluated

Non-activated 4-hour exposure group:	Concentrations of 100, 400, and 1550 µg IN-PLT97/mL in duplicate in the absence of S9 activation.
S9-activated 4-hour exposure group:	Concentrations of 50, 100 and 500 µg IN-PLT97/mL in duplicate in the presence of S9 activation.
Non-activated 20-hour exposure group:	Concentrations of 100, 400, and 1500 µg IN-PLT97/mL in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

June 24, 2010 to August 17, 2010

2. Preliminary cytotoxicity assay

The toxicity test was performed for the purpose of selecting concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, number of dividing cells/1000 cells counted). The cells were exposed to solvent alone and to concentrations of the test substance ranging from 0.155 to 1550 µg/mL for 4 hours in both the presence and absence of S9 activation and for 20 hours continuously in the absence of S9 activation.

3. Cytogenetic assay

Cell treatment: Cells were exposed to test compound, solvent or positive control for 4 h or 20 h (non-activated) or 4 h (activated).

Treatment condition	Treatment time (h)	Recovery time (h)	Concentrations (µg/mL)
Non-activated	4	16	50, 100, 200, 400, 800, 1550
	20	0	25, 50, 100, 200, 500, 1000, 1550
S9 activated	4	16	50, 100, 200, 400, 800, 1550

Spindle inhibition: Two hours prior to the scheduled cell harvest at 20 h after treatment initiation, Colcemid® was added to the cell cultures at a final concentration of 0.1 µg/mL.

Cell harvest: Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation at 1200 rpm for about 5 minutes. The cell pellet was resuspended in 5 mL of 0.075 M KCl and incubated for 20 minutes. At the end of the KCl treatment and immediately prior to

centrifuging, the cells were gently mixed and approximately 0.5 mL of fixative (methanol:glacial acetic acid, 3:1 v/v) was added to each tube. The cells were collected by centrifugation, the supernatant aspirated, and the cells were fixed with two washes with approximately 3 to 5 mL of fixative and stored in fixative overnight or longer at approximately 2 to 8°C.

Slide preparation: Fixed cells were centrifuged at approximately 1200 rpm for 5 minutes. The supernatant was aspirated, leaving 0.1 to 0.3 mL fixative above the cell pellet. An aliquot of the cell suspension was dropped onto a glass slide and allowed to air dry at room temperature. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Selection of Dose Levels for Analysis: The selection of dose levels for analysis of chromosome aberrations in HPBL was based upon toxicity of the test substance. The highest dose level selected for evaluation was the dose which induced at least 50% toxicity, as measured by mitotic inhibition, relative to the solvent control, with a sufficient number of scorable metaphase cells. In the absence of ≥ 50% toxicity, the highest dose level evaluated was the highest dose tested in the chromosome aberration assay. Two additional lower dose levels were included in the evaluation.

Evaluation of metaphase cells: Metaphase cells with 46 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase-spreads (100 per replicate treatment condition) were examined for chromatid-type and chromosome-type aberrations. Chromatid type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverised chromosome(s), pulverised cells and severely damaged cells (≥10 aberrations) also were recorded. Chromatid gaps and isochromatid gaps were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using the microscope stage. The percent polyploid and endoreduplicated cells were evaluated per 100 cells.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose-responsiveness.

5. Evaluation criteria

The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent negative control

group ($p \leq 0.05$). However, values that are statistically significant, but do not exceed the range of historical solvent controls may be judged as not biologically significant. Test substances not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

IN-PLT97 was present at acceptable concentrations in the dosing solutions (96.4% of nominal concentrations). IN-PLT97 was shown to be stable in the dosing solutions under the conditions of the study. IN-PLT97 was not found in the 0 mg/mL samples. The positive and solvent controls fulfilled the requirements for a valid test.

Dosing formulations were adjusted for the initial Sponsor-reported purity (96.7%), using a correction factor of 1.034. However, the Certificate of Analysis reports a purity of 98.1% active ingredient. All subsequent dose levels cited represent the nominal values based the initially reported purity, and are consequently 101.4% of the intended dose levels based on the purity stated on the Certificate of Analysis.

B. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance formed a workable suspension in DMSO at a maximum concentration of approximately 100 mg/mL. Visible precipitate was observed in treatment medium at concentrations ≥465 μg/mL. Toxicity (mitotic inhibition) in excess of 50%, relative to the solvent control, was not observed at any concentration in the non-activated 4-hour and 20-hour exposure groups; toxicity was observed at 1550 μg/mL in the S9-activated 4-hour exposure group (Table 1).

C. CHROMOSOME ABERRATION ASSAY

In the chromosome aberration assay, visible precipitate was observed in treatment medium at ≥400 μg/mL and dose levels ≤200 μg/mL were soluble in treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, in the non-activated 4-hour exposure group, visible precipitate was observed in treatment medium at ≥200 μg/mL and dose levels ≤100 μg/mL were soluble in treatment medium; in the activated 4-hour exposure group, visible precipitate was observed in treatment medium at ≥500 μg/mL and dose levels ≤200 μg/mL were soluble in treatment medium. In the non-activated 20-hour exposure group, visible precipitate was observed in treatment medium at ≥ 200 μg/mL and dose levels ≤100 μg/mL were soluble in treatment medium. The summary and the details of the data are presented in Table 2.

Non-activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 1550 μg/mL, mitotic inhibition was 24%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test

substance-treated groups was not significantly increased above that of the solvent control (p >0.05, Fisher's exact test).

Activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 500 μg/mL, mitotic inhibition was 63%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control (p >0.05, Fisher's exact test).

Non-activated 20 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 1550 μg/mL, mitotic inhibition was 25%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control (p >0.05, Fisher's exact test).

III. CONCLUSIONS

IN-PLT97 was negative for structural and numerical chromosome aberrations in the non-activated and S9-activated test systems in the *in vitro* mammalian chromosome aberration test using human peripheral blood lymphocytes.

Table 1. Summary results of toxicity the toxicity test
(Data excerpted from pages 23-25 of the report)

4-HOUR TREATMENT		
Treatment (-S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	12.8	
IN-PLT97		
0.155	13.2	3
0.465	13.6	6
1.55	13.6	6
4.65	13.0	2
15.5	13.4	5
46.5	13.4	5
155	12.8	0
465	13.0	2
1550	11.0	-14

4-HOUR TREATMENT		
Treatment (+S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	12.6	
IN-PLT97		
0.155	12.4	-2
0.465	13.0	3
1.55	11.8	-6
4.65	12.2	-3
15.5	13.0	3
46.5	12.2	-3
155	12.6	0
465	6.4	-49
1550	5.8	-54

20-HOUR TREATMENT		
Treatment (-S9) µg/mL	Mitotic Index (%)	Percent Change (%)
DMSO	13.6	
IN-PLT97		
0.155	13.0	-4
0.465	13.8	1
1.55	13.6	0
4.65	12.8	-6
15.5	13.0	-4
46.5	12.8	-6
155	12.6	-7
465	13.0	-4
1550	13.6	0

Treatment: Human peripheral blood lymphocyte cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.
Mitotic Index = (Cells in mitosis/500 cells scored) x 100.
Percent change = (Treatment mitotic index - control mitotic index)/control mitotic index, expressed as a percentage.

Table 2. Summary of chromosome aberration data for 4 or 20 hours exposures in the presence or absence of S9- activation
(Table excerpted from page 29 of the study report).

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	15.2	200	200	0.000	±0.000	0.0	0.0
IN-PLT97									
100	-S9	4	14.6	200	200	0.000	±0.000	0.5	0.0
400	-S9	4	13.0	200	200	0.005	±0.071	0.5	0.5
1550	-S9	4	11.6	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	4	10.9	200	100	0.280	±0.552	0.0	24.0**
DMSO	+S9	4	13.4	200	200	0.005	±0.071	0.0	0.5
IN-PLT97									
50	+S9	4	13.0	200	200	0.005	±0.071	0.5	0.5
100	+S9	4	12.5	200	200	0.000	±0.000	0.0	0.0
500	+S9	4	5.0	200	200	0.015	±0.122	0.0	1.5
CP, 10	+S9	4	4.1	200	50	0.420	±0.499	0.0	42.0**
DMSO	-S9	20	14.8	200	200	0.000	±0.000	0.0	0.0
IN-PLT97									
100	-S9	20	13.6	200	200	0.000	±0.000	0.5	0.0
400	-S9	20	11.9	200	200	0.000	±0.000	0.0	0.0
1550	-S9	20	11.1	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	5.6	200	50	0.380	±0.635	0.0	30.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.

Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIA 5.8/05

Report: Clarke, J.J. (2010); IN-PLT97: *In vitro* mammalian cell gene mutation test (CHO/HGPRT Assay). BioReliance, Rockville, Maryland, USA. Testing Laboratory Report No.**:** AD08NK.782.BTL. DuPont Report No.: DuPont-30365. November 10, 2010. MRID 48122582. Unpublished.

Guidelines: OPPTS 870-5300
EU 2000/32/EC, Annex 4E No. L136
OECD 476 (1998)
JMAFF 59-Nousan-4200 (1985)
Deviations: None

GLP: Yes Signed and dated statements of GLP Compliance, Quality Assurance, and Data Confidentiality were presneted in the report.

Executive summary:

In the CHO/HGPRT mutation assay (MRID 48122582), IN-PLT97 (98.1% purity) was tested with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K_1-BH_4 cells were exposed for 5 hours at 37 ± 1°C to the test substance at concentrations of 10, 25, 50, 100, and 150 μg/mL. The highest dose level was set based on insolubility of the test substance at concentrations ≥ 150 μg/mL. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-thioguanine (2-amino-6-mercaptopurine)-resistant phenotype. The test substance was dissolved in dimethylsulfoxide (DMSO) at a maximum concentration of 150 mg/mL. Ethyl methanesulfonate (EMS) and Benzo(a)pyrene (B(a)P) were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of ≤50% of the concurrent vehicle control. The assay was considered positive when a dose-dependent increase in mutation frequencies occurred with at least two consecutive doses having mutation frequencies of greater than 40 mutants per 10^6 clonable cells.

In the mutagenesis assay, no positive responses were observed. Visible precipitate was seen at ≥100 μg/mL. Mile toxicity was observed (relative cloning efficiency 82% and 80%) at the highest dose tested in the non-activated and S-9 activated systems, respectively. **Therefore, IN-PLT97 was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.**

This study is fully reliable (acceptable/guideline) and satisfies the requirement for an *In vitro* mammalian cell gene mutation assay (OPPTS 870-5300; OECD 476).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-PLT97 technical metabolite
   Lot/Batch #: PLT97-003
   Purity: 98.1%
   Description: White powder
   CAS #: Not available
   Stability of test compound: Results from analysis of the dosing solutions indicated that the test substance was present at acceptable concentrations and stable under the conditions of the study.
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)
   Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 0.2 µL/mL
   Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4 µg/mL
3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2568
   Source: Moltox, Boone, NC
   Protein content: 33.5 mg/mL
   Characterisation: The metabolic activation ability of the S9 was characterised using varying S9 and positive control concentrations.

   S9 mix composition
   NADP: 4 mM
   Glucose-6-phosphate: 5 mM
   KCl: 30 mM
   $MgCl_2$: 10 mM
   Sodium phosphate buffer (pH 7.5): 50 mM
   Calcium chloride: 10 mM
   S9 homogenate: 10% (v/v)

4. Test cells

   Chinese Hamster Ovary cells (CHO-K1) were properly maintained, periodically checked for mycoplasma contamination, and periodically "cleansed" against high spontaneous background. Cells used in the mutation assay were within four subpassages from cleansing in order to assure karyotypic stability.

5. Culture medium

   F12FBS5-Hx (Ham's F12 medium without hypoxanthine supplemented with 5% foetal bovine serum (FBS), 100 units penicillin/mL, 100 μg streptomycin/mL, and 2 mM L-glutamine/mL.

6. Locus examined

   Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) – the selection agent used was 10 μM 6-thioguanine (6TG, 2-amino-6-mercaptopurine).

7. Test compound concentrations used

   Preliminary cytotoxicity

   Trial 1: Concentrations of 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, and 1000 μg IN-PLT97/mL were evaluated in duplicate in the presence and absence of S9 activation.

   Mutagenesis assay

   Trial 1: Concentrations 10, 25, 50, 100, and 150 μg IN-PLT97/mL were evaluated in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   23-June-2010 to 12-August-2010

2. Preliminary cytotoxicity assay

   CHO cells were exposed for 5 hours to vehicle alone and nine concentrations of test substance ranging from 0.5 to 5000 μg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenesis assay

   CHO cells were exposed for 5 hours to the vehicle alone, appropriate positive controls, and six concentrations of test substance in duplicate in both the absence and presence of S9-activation. After 5 hours, the cells were washed with Ca^{++} and Mg^{++} – free Hanks' balanced salt solution (HBSS) and cultured for an additional 18–24 hours. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

   Cytotoxicity: The replicates from each treatment condition were detached using trypsin and subcultured in triplicate at a density of 200 cells/60 mm dish. After 7 to 10 days incubation, the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted and cloning efficiency determined.

Phenotypic expression/selection: The replicates from each treatment condition were detached using trypsin and subcultured in duplicate at a density no greater than 10^6 cells/100 mm dish. Subculturing at 2 to 4 day intervals was employed for the 7 to 9 day expression period. For selection of the TG-resistant phenotype, the replicates from each treatment condition were trypsinised and replated, in quintuplicate, at a density of 2×10^5 cells/100 mm dish in medium containing 10 μM TG. For cloning efficiency determination at the time of selection, 200 cells/60 mm dish were plated in triplicate. After 7 to 10 days of incubation, the colonies were fixed, stained and counted for both cloning efficiency and mutant selection.

4. Statistics

The data did not warrant statistical analysis.

5. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies with at least two consecutive doses showing mutant frequencies of greater than 40 mutants per 10^6 clonable cells. If no culture exhibited a mutant frequency of greater than 40 mutants per 10^6 clonable cells, the test substance was considered negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

PLT97 was present at acceptable concentrations in the dosing solutions (5.3% of nominal concentrations). IN-PLT97 was shown to be stable in the dosing solutions under the conditions of the study. IN-PLT97 was not found in the 0 mg/mL samples. The positive and solvent controls fulfilled the requirements for a valid test.

Dosing formulations were adjusted for the initial Sponsor-reported purity (96.7%), using a correction factor of 1.034. However, the Certificate of Analysis reports a purity of 98.1% active ingredient. All subsequent dose levels cited in this report represent the nominal values based the initially reported purity, and are consequently 101.4% of the intended dose levels based on the purity stated on the Certificate of Analysis.

B. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. After sonication, the test substance was workable in DMSO at concentrations from approximately 50 to 100 mg/mL. There was visible precipitate in the treatment medium at test substance concentrations ≥150 μg/mL. No visible precipitate was observed at concentrations ≤50 μg/mL. Cloning efficiency at 1000 μg/mL was 79% without activation and 64% with activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 5.0 to 150 μg/mL for both the non-activated and S9-activated cultures.

Table 1. Preliminary toxicity results
(Table excerpted from page 17 of the study report)

Treatment (µg/mL)	PREC IP	NON-ACTIVATED					S9-ACTIVATED				
		Plate Counts			Cloning Efficiency (%)	Relative Cloning Efficiency (%)	Plate Counts			Cloning Efficiency (%)	Relative Cloning Efficiency (%)
		1	2	3			1	2	3		
Solvent		158	127	136	70	**100**	118	132	129	63	**100**
0.15		104	122	134	60	**86**	111	102	143	59	**94**
0.5		124	129	117	62	**88**	113	99	100	52	**82**
1.5		125	121	144	65	**93**	88	117	72	46	**73**
5		125	122	119	61	**87**	118	140	112	62	**98**
15		26	35	38	17	**24**	97	106	92	49	**78**
50		21	17	33	12	**17**	95	96	121	52	**82**
150	P	109	84	94	48	**68**	118	97	87	50	**80**
500	P	110	92	110	52	**74**	94	55	82	39	**61**
1000	P	104	95	132	55	**79**	66	85	90	40	**64**

Solvent = DMSO
P – Precipitating concentration

$$\text{Cloning efficiency} = \frac{\text{total colonies counted}}{\text{number of dishes x 200 cells/dish}}$$

$$\text{Relative cloning efficiency} = \frac{\text{cloning efficiency of treatment group}}{\text{cloning efficiency of solvent group}} \times 100$$

B. MUTAGENESIS ASSAY

Table 2 presents the summarized results of the mutation assay, while Table 3 and 4 provide the details of the test. There was visible precipitate in the treatment medium at test substance concentrations ≥100 µg/mL. Relative cloning efficiency was 82% and 80% at 150 µg/mL, the highest dose tested, in the non-activated and S9-activated systems, respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 10^6 clonable cells.

Table 2. Summary of mutagenicity findings
(Data extracted from Table s 3 & 4 on pages 19 and 20 of the study report)

Compound	Conc. µg/mL	Mutation frequency without activation[a]	Mutation frequency with activation[a]
IN-PLT97	0	11.8	0
	10	3.4	11.4
	25	10.8	8.8
	50	0.7	2.5
	100	9.4	0
	150	0.8	0
EMS	-	408.8	Ne
B(a)P	-	ne	122.4

[a] Mutants per 1×10^6 surviving cells defined as: total mutant colonies/(number of selection dishes x cloning efficiency $\times 2 \times 10^5$ cells) $\times 10^6$

EMS = ethyl methanesulfonate, 0.2 µL/mL B(a)P = benzo(a)pyrene, 4 µg/mL
ne = Not evaluated

Table 3. Non-activated (-S9) mutation assay reults
(Table excerpted from page 19 of the study report)

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates				Cloning Efficiency (%)	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells	Relative Cloning Efficiency (%)
			Plate Counts			Average Colonies		Plate Counts					Average Colonies		
			1	2	3			1	2	3	4	5			
Solvent	A		159	144	163	152.0	76	0	0	0	0	0	1.8	11.8	100
	B		152	135	159			4	4	4	2	4			
EMS (0.2µL/mL)	A		103	80	70	83.2	42	29	25	40	47	33	34.0	408.8	50
	B		66	87	93			45	40	37	22	22			
10	A		140	129	157	146.7	73	0	0	0	0	0	0.5	3.4	92
10	B		185	163	106			0	3	2	0	0			
25	A		125	121	116	138.7	69	0	1	5	1	8	1.5	10.8	85
25	B		158	150	162			0	0	0	0	0			
50	A		123	117	108	152.3	76	0	0	0	0	1	0.1	0.7	103
50	B		164	169	233			0	0	0	0	0			
100	A		148	157	172	148.7	74	1	4	1	1	2	1.4	9.4	120
100	B		124	170	121			1	2	0	1	1			
150	A	P	120	194	136	130.8	65	0	0	0	0	0	0.1	0.8	82
150	B	P	109	120	106			0	0	0	1	0			

Table 4. Activated (+S9) mutation assay results.
(Table excerpted from page 20 of the study report)

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates				Cloning Efficiency (%)	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells	Relative Cloning Efficiency (%)
			Plate Counts			Average Colonies		Plate Counts					Average Colonies		
			1	2	3			1	2	3	4	5			
Solvent	A		173	131	127	147.8	74	0	0	0	0	0	0	0	100
	B		189	139	128			0	0	0	0	0			
B(a)P (4µg/mL)	A		119	132	123	131.5	66	15	14	23	26	10	16.1	122.4	55
	B		132	136	147			9	13	30	17	4			
10	A		157	147	167	139.8	70	2	0	0	0	0	1.6	11.4	83
10	B		121	138	109			0	4	1	6	3			
25	A		107	113	121	137.0	69	1	0	0	0	0	1.2	8.8	78
25	B		143	149	189			1	1	4	5	0			
50	A		122	102	146	118.7	59	0	0	0	1	0	0.3	2.5	81
50	B		103	115	124			0	0	2	0	0			
100	A		94	124	143	123.7	62	0	0	0	0	0	0	0	82
100	B		130	86	165			0	0	0	0	0			
150	A	P	81	105	104	103.2	52	0	0	0	0	0	0	0	80
150	B	P	98	109	122			0	0	0	0	0			

Solvent = DMSO
A and B are duplicate cultures
P – Precipitating concentration

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{200 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

III. CONCLUSION

Under the conditions of this mutagenesis assay, no positive responses were observed. Visible precipitate was seen at ≥100 μg/mL. Mile toxicity was observed (relative cloning efficiency 82% and 80%) at the highest dose tested in the non-activated and S-9 activated systems, respectively. **Therefore, IN-PLT97 was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.**

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.1/01	***In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay**
Report:	Wagner, V.O. VanDyke, M.R. (2010); Cyantraniliprole (DPX-HGW86) technical: Bacterial reverse mutation assay, DuPont-30991. BioReliance, Rockville, Maryland, USA **Report No.:** AD10PN.503.BTL. MRID 48122587
Dates of work:	17 August-2010 to 07-Setember 2010
Guidelines:	**Guidelines:** OPPTS 870.5100 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: Yes (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In independent trials of a reverse gene mutation assay (MRID 48122587), cyantraniliprole (95.6% Lot No. D100487-104) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) in two phases using the plate incorporation method. Nominal concentrations were 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate in the toxicity-mutation phase (trial I). Based on the toxicity-mutation test, the dose levels tested in the mutagenicity test (trial II) were 0, 50, 150, 500, 1500, and 5000 μg/plate. Dose levels were evaluated using standard plate incorporation methods. The highest dose level in the mutagenicity phase was set based on the results of the toxicity-mutation phase. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL.

Precipitate was observed at 5000 μg per plate in both trials. No appreciable cytotoxicity was seen in either trial. The positive and vehicle controls fulfilled the requirements for a valid test. The number of revertants at all concentrations of the test substance was similar to concurrent controls in trials both with and without activation.

Under the conditions of this study, cyantraniliprole was negative for mutagenic activity in non-activated and S9-activated test systems.

The study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: D100487-104
   Purity: 95.6%
   Description: Solid powder
   CAS # 736994-63-1
   Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.) Dosing formulations were adjusted to 100% purity using a correction factor of 1.055
2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)/50 μL

Positive control, non activation:

Positive control	Dose	Strain(s)
2-Nitrofluorene	1 µg/plate	TA98
Sodium azide	1 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-Aminoanthracene	1 µg/plate	TA98, TA1535, TA1537
	2 µg/plate	TA100
	10 µg/plate	WP2 *uvr*A

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2639, 2648
   Source: Moltox, Inc., Boone, NC
   Protein content: 34.0 and 41.5 mg/mL
   Source: Not applicable
   Characterisation: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.
   S9 mix composition:
   Phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM
   $MgCl_{2:}$ 8 mM
   S9: 10% (v/v)

4. Test organisms

   Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

   Exp. No. B1: (Initial Toxicity-Mutation Assay) Concentrations of 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg cyantraniliprole/plate were evaluated in duplicate in the presence and absence of S9 activation.

   Exp. No. B2: (Confirmatory Mutagenicity Assay) Concentrations of 0, 50, 150, 500, 1500 and 5000 µg cyantraniliprole/ plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

17-August -2010 to 07-September-2010

2. Plate incorporation assay

This study consisted of 2 independent trials: a preliminary toxicity-mutation phase (trial I) and a mutagenicity phase (trial II). In both trials, 0.5 mL of S9 or Sham mix, 100 µL of tester strain (cells seeded) and 50 µL of vehicle or test substance dilution were added to 2.0 mL of molten selective top agar at 45 ± 2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37 ± 2°C. Plates that were not counted immediately following the incubation period were stored at 2 to 8°C until colony counting could be conducted. All toxicity-mutation test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in duplicate. All mutagenicity test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in triplicate.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Toxicity was scored relative to the concurrent vehicle control plates and recorded with the mean revertant count for the strain, condition, and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter.

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

A test substance was classified as positive when the mean number of revertants in any strain except TA1535 and TA1537 and at any test substance concentration was at least 2 times greater than the mean number of revertants in the concurrent vehicle control and occurred in a positive dose-response relationship. For strains TA1535 and TA1537 a mean number of revertants of at least 3 times greater than vehicle control was needed to be considered a positive response. The performing laboratory provided historical control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (± 11.0% of nominal concentrations). Cyantraniliprole was shown to be stable in the dosing solutions under the conditions of the study. Cyantraniliprole was not found in the 0 mg/mL samples.

B. MUTATION ASSAYS

Compound precipitation was observed at 5000 µg per plate in both trials. No test substance-related toxicity was observed in either trial as is evidenced by a reduction of the microcolony background lawns and/or by a concentration-related decrease in mean revertants per plate. All positive controls exhibited more than a 3-fold increase in mean revertants over the respective mean of the vehicle controls. For all strains except TA1535 and TA1537, no test substance concentration produced a mean 2 times greater than the mean of its respective negative control. For strains TA1535 and TA1537, no test substance concentration produced a mean 3 times

greater than the mean of its respective negative control. There was no concentration-related increase in the mean revertants per plate in any strain in either trial (Tables 1 and 2).

Controls: The positive and vehicle controls fulfilled the requirements for a valid test.

Table 1
Summary of average revertants/plate without activation of cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I[a]	Trial II[b]	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Cyantraniliprole	0	14	21	105	112	17	16	15	7	30	31
	1.5	20	ne	104	ne	17	ne	18	ne	36	ne
	5.0	21	ne	116	ne	17	ne	9	ne	45	ne
	15	13	ne	109	ne	17	ne	15	ne	33	ne
	50	15	12	107	107	23	17	7	6	39	36
	150	17	14	109	102	15	21	15	8	27	31
	500	21	19	119	97	13	21	13	6	37	34
	1500	21	13	126	118	15	18	17	8	28	25
	5000	15	19	108	114	13	17	16	4	36	30
Positive control											
2NF	1	247	211	ne	ne	ne	ne	ne	ne	ne	ne
NAAZ	1	ne	ne	798	894	726	722	ne	ne	ne	ne
9AA	75	ne	ne	ne	ne	ne	ne	2035	705	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	589	556

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

ne = Not evaluated

2NF = 2-nitrofluorene; NAAZ = Sodium azide; 9AA = 9-aminoacridine; MMS = Methyl methanesulfonate;

ne = Not evaluated

Data were derived from Tables 1 and 2, pp. 20, 21, 24 and 25, MRID 48122587.

Table 2
Summary of average revertants/plate with activation of cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Trial I [a]	Trial II [b]	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Cyantraniliprole	0	18	21	114	110	8	11	8	8	42	43
	1.5	21	ne	109	ne	10	ne	14	ne	26	ne
	5.0	25	ne	98	ne	11	ne	9	ne	44	ne
	15	22	ne	120	ne	9	ne	9	ne	37	ne
	50	28	25	112	115	9	12	10	5	35	46
	150	26	20	123	118	7	12	10	10	56	42
	500	20	20	125	112	14	9	11	8	44	38
	1500	25	26	131	107	13	13	11	8	44	42
	5000	19	23	113	121	11	13	14	8	42	42
Positive control											
2AA	1	375	357	ne	ne	87	105	41	73	ne	ne
	2	ne	ne	355	737	ne	ne	ne	ne	ne	ne
	10	ne	ne	ne	ne	ne	ne	ne	ne	132	244

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

ne = Not evaluated

Data were derived from Tables 3 and 4, pp. 22, 23, 26, and 27, MRID 48122587.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 471 (1997)

C. CONCLUSIONS: Cyantraniliprole was negative for mutagenic activity up to precipitating concentrations in all strains in both trials in the presence and absence of S9-activation in the *in vitro* bacterial gene mutation assay. There were no treatment-related increases in the mean number of revertants/plate in any strain (+/-S9). The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

Accordingly, Cyantraniliprole is negative in this test system in a well-done study.

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Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)
IIA 5.4.2/03 ***In vitro* Mammalian Chromosome Aberration Test**

Report:	Madraymootoo, W., Jois, M. (2010); Cyantraniliprole (DPX-HGW86) technical: *In vitro* mammalian chromosome aberration test. DuPont-30990; BioReliance, Rockville, Maryland, USA, Report No.: AD10PN.341.BTL (MRID 48122588).
Dates of work:	12-August-2010 to 23-September-2010
Guidelines:	OPPTS 870.5375 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: NO (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.
GLP: Exceptions	No≈

Executive Summary:

Cyantraniliprole (95.6%; Lot No. D100487-104) was tested in the *in vitro* mammalian chromosome aberration test (MRID 48122588) using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). In the preliminary toxicity assay, the cells were exposed to nine concentrations of the test substance ranging from 0.3461 to 3461 μg/mL, as well as a control vehicle. Visible precipitate was observed in treatment medium at levels ≥3461.1 μg/mL and doses ≤103.83 μg/mL were soluble in treatment medium at the beginning of the test period. Substantial toxicity [at least 50% reduction in mitotic index (MI) relative to the solvent control] was observed at 1038.3 μg/mL in the non-activated 4-hour exposure group, at ≥1038.3 μg/mL in the S9-activated 4-hour exposure group, and at 346.1 and 1038 μg/mL in the non-activated 20-hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay were 0, 62.5, 125, 250, 500, 600, 700, 800, and 1000 μg/mL for non-activated 4-hour; 0, 62.5, 125, 250, 500, 600, 700, 800, and 900 μg/mL for activated 4-hour; and 0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 μg/mL for non-activated 20-hour. The test substance formed a workable suspension in dimethyl sulfoxide (DMSO) at a concentration of 346.1 mg/mL, the maximum concentration prepared for the preliminary toxicity assay and was soluble in DMSO at all concentrations tested in the definitive chromosome aberration assay. HPBL were treated for 4 hours (activated and non-activated test system), and 20 hours (non-activated test system). After exposure to Colcemid®, metaphase cells were harvested approximately 20 hours following the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition) then structural and numerical chromosome aberrations

In the chromosome aberration assay, visible precipitate was observed in treatment medium at ≥500 μg/mL and levels ≤250 μg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. At the highest concentrations examined for chromosome aberrations [800 and 600 μg/mL without or with S9 activation, respectively (4-h treatment) or 250 μg/mL without S9 (20-h treatment)], MIs were generally reduced by 50-57%. The positive and solvent controls fulfilled the requirements for a valid test. However, no statistically significant increases in structural chromosome aberrations were observed in either trial at any of the concentrations evaluated. In addition, no statistically significant increases in polyploidy were observed. Positive controls induced the appropriate response.

Based on the findings of this study, cyantraniliprole was concluded to be negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes with and without an exogenous metabolic activation system.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for an i*n vitro* mammalian cytogenetics (chromosome aberrations) assay (OPPTS 870.5375; OECD 473).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: D100487-104
   Purity: 95.6%
   Description: Solid, white powder
   CAS # 736994-63-1
   Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study. Dose formulations were adjusted to 100% purity using a correction factor of 1.055.

2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)
   Positive, non-activation: Mitomycin C (MMC) in water at 0.3 and 0.6 µg/mL
   Positive, activation: Cyclophosphamide (CP) in water at 10 µg/mL

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2648
   Source: Moltox, Inc., Boone, NC
   Protein content: 41.5 mg/mL
   Characterisation: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition
   Glucose-6-phosphate: 1 mM
   NADP: 1 mM
   KCl: 6 mM
   $MgCl_{2:}$ 2 mM
   S9: 20 µL per mL medium

4. Test cells

   Human lymphocytes were obtained from the peripheral blood of a healthy, non-smoking adult female and were cultured in RPMI 1640 supplemented with 1% phytohemagglutinin (PHA). Cultures were incubated for 44-48 hours prior to testing.

5. Culture medium (for the assays)

   RPMI 1640, serum-free medium containing 15% fetal bovine serum, antibiotics and 2 mM L-glutamine

6. Test compound concentrations evaluated

   Preliminary cytotoxicity test: 0, 0.3461, 1.038, 3.461, 10.383, 34.61, 103.83, 346.1, 1038.3, and 3461 µg cyantraniliprole/mL +/-S9.

Chromosome aberration assay:

Non-activated 4-hour exposure group	0, 125, 250, and 800 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.
S9-activated 4-hour exposure group	0, 250, 500, and 600 µg cyantraniliprole/mL in duplicate in the presence of S9 activation.
Non-activated 20-hour exposure group	0, 31.3, 62.5, and 250 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

12-August-2010 to 23-September-2010

2. Treatment:

Preliminary cytotoxicity assay: The toxicity test was performed to select concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, the percentage of cells in mitosis per 500 cells counted). The cells were exposed to solvent alone and to nine concentrations of the test substance for 4 hours in both the presence and absence of S9 activation, and for 20 hours continuously in the absence of S9 activation.

Cytogenetic assay: Independently performed experiments were conducted

a. Cell exposure time:

	Test material	Solvent control	Positive control
Non-activated:	4 h 20 h	4 h 20 h	4 h 20 h
Activated:	4 h	4 h	4 h

b. Spindle inhibition:

Inhibition used/concentration:	Colcemid/ 0.1 µg/mL
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

	Test material	Solvent control	Positive control
Non-activated and Activated:	16 or 0 h & 16 h	16 or 0 h & 16 h	16 or 0 h & 16 h

d. Details of slide preparation: Following the 2-hour colcemid treatment, cells were treated with hypotonic KCl (0.075 M) for 20 min at 37° C. Cells were then fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5 % Giemsa. Two slides per group per experiment were prepared.

e. Metaphase analysis

No. of cells examined per dose: 200 (100 per duplicate culture) were scored.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy cells		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent negative control group ($p \leq 0.05$). However, values that are statistically

significant but do not exceed the range of historical solvent controls may be judged as not biologically significant. The performing laboratory provided historical control data.

g. Statistical analysis: Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose-responsiveness.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance formed a workable suspension at 346.1 mg/mL, the maximum concentration tested in the preliminary toxicity assay and was soluble in DMSO at all concentrations tested in the definitive chromosome aberration. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (0.75, 25, and 100 mg/mL within 102.8%, 96.4%, and 99.4% of nominal concentrations, respectively). Cyantraniliprole was shown to be stable in the dosing solutions under the conditions of the study. Cyantraniliprole was not found in the 0 mg/mL samples. The test material at the lowest precipitating dose (346.1 µg/mL) had no adverse effect on the pH or osmotic pressure of the culture medium.

B. PRELIMINARY CYTOTOXICITY ASSAY

Visible precipitate was observed in treatment medium at concentrations ≥ 346.1 µg/mL and levels ≤ 103.83 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was observed at 1038.3 µg/mL in the non-activated 4-hour exposure group, at dose levels ≥ 1038.3 µg/mL in the S9-activated 4-hour exposure group, and at least dose levels 346.1 and 1038 µg/mL in the non-activated 20-hour exposure group. Based on these results, concentrations presented below in Table 1 were selected for the chromosome aberration assay:

Table 1. Dose selection for the chromosome aberrations assay with cyantraniliprole

Treatment condition	Treatment time (h)	Concentrations (µg/mL)
Non-activated	4	0, 62.5, 125, 250, 500, 600, 700, 800, 1000
	20	0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000
S9 activated	4	0, 62.5, 125, 250, 500, 600, 700, 800, 900

C. CHROMOSOME ABERRATION ASSAY

In the chromosome aberration assay, visible precipitate was observed in treatment medium at concentrations ≥ 500 µg/mL and dose levels ≤ 250 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Summarized results from the chromosome aberration assays are presented in Study Report Table 7 and are as follows:

Non-activated 4 h exposure group: At the highest test concentration evaluated microscopically for chromosome aberrations, 800 µg/mL, mitotic inhibition was 53%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test).

Activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 600 µg/mL, mitotic inhibition was 57%, relative to the solvent control. The percentage of cells with structural and

numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control (p >0.05, Fisher's exact test).

Non-activated 20 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 250 µg/mL, mitotic inhibition was 50%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control (p >0.05, Fisher's exact test).

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable (EPA rating Acceptable/guideline)

This study is fully compliant with OECD 473 (1997)

C. CONCLUSIONS: Cyantraniliprole was tested up to concentrations [800 µg/mL–S9 or 600 µg/mL +S9 (4-hour treatment) and 250 µg/mL -S9 (20-hour treatment)] that were either insoluble or reduced the MI by ≈50% but failed to induce a clastogenic effect. The expected responses were obtained with the negative and positive controls either with or without S9 activation at both time intervals. Based on these considerations, it was, therefore, concluded that cyantraniliprole was negative in this test system in a well-conducted study.

Deficiencies: None

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	12.7	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
125	-S9	4	11.8	200	200	0.000	±0.000	0.0	0.0
250	-S9	4	11.8	200	200	0.000	±0.000	0.0	0.0
800	-S9	4	6.0	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	2.7	200	100	0.190	±0.465	0.0	16.0**
DMSO	+S9	4	9.3	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
125	+S9	4	8.5	200	200	0.000	±0.000	0.0	0.0
250	+S9	4	8.8	200	200	0.000	±0.000	0.0	0.0
600	+S9	4	4.0	200	200	0.000	±0.000	0.0	0.0
CP, 10	+S9	4	2.5	200	100	0.120	±0.327	0.0	12.0**
DMSO	-S9	20	11.7	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
31.3	-S9	20	9.5	200	200	0.000	±0.000	0.0	0.0
62.5	-S9	20	8.9	200	200	0.000	±0.000	0.0	0.0
250	-S9	20	5.9	200	200	0.005	±0.071	0.0	0.5
MMC, 0.3	-S9	20	3.2	200	100	0.180	±0.479	0.0	15.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.
All dose levels tested in the chromosome aberration assay were 91% of nominal.

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

Report: IIA 5.4.3/01	Clarke, J.J. (2010); Cyantraniliprole (DPX-HGW86) technical: In vitro mammalian cell gene mutation test (CHO/HGPRT) assay. DuPont-30992 BioReliance, Rockville, MD, Report No.: AD10PN.782.BTL (MRID 48122589)
Dates of work:	18-August-2010 to 16 September-2010
Guidelines:	OPPTS 870.5300 (1998) OECD 476 (1997) UKEMS Guidelines (1990) JMAFF 12-Nousan-8147 (2000) JMAFF, draft
GLP: Yes (certified laboratory)	OECD Principles of GLP, ENV/MC/CHEM (1998) Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

Cyantraniliprole (95.6%; Lot No. D100487-104) was tested in the CHO/HGPRT mutation assay (MRID 48122589) with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). The preliminary toxicity assay was used to establish the dose range for the mutagenesis assay. Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K_1-BH_4 cells were exposed for 5 hours at 37 ± 1°C to the test substance at concentrations of 0, 50.0, 100, 150, 250, and 500 µg/mL, in both the absence and presence of S9. The highest dose level was set based on cloning efficiency and the precipitation profile in a preliminary toxicity assay. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-thioguanine (2-amino-6-mercaptopurine) -resistant phenotype. Ethyl methanesulfonate (EMS) and benzo(a)pyrene (BaP) were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of ≤50% of the concurrent vehicle controls.

In the mutation assay, test material precipitation was recorded at 500 µg/mL+/-S9. Relative cloning efficiencies at this level were 70%(-S9) and 83% (+S9). The positive and solvent controls induced the expected response. However, there were no statistically significant or dose-dependent increases in the average mutant frequent observed with or without S9.

It was concluded, therefore, that Cyantraniliprole was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.

This study is classified as **totally reliable** (**acceptable/guideline)** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: D10087-104
   Purity: 95.6%
   Description: Solid, powder
   CAS #: 736994-63-1
   Stability of test compound: The test substance was at the targeted concentrations and stable when stored at room temperature during the dosing period in the vehicle for all dose levels. Dose formulations were adjusted to 100% purity using a correction factor of 1.055.

2. Control materials
   Solvent control: Dimethylsulfoxide (DMSO)
   Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 0.2 µL/mL
   Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4 µg/mL

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2568
   Source: Molecular Toxicology, Inc., Boone, NC
   Protein content: 33.5 mg/mL
   Characterization: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.

S9 mix composition (final concentration in cultures):

NADP:	4 mM
Glucose-6-phosphate:	5 mM
KCl:	30 mM
$MgCl_{2:}$	10 mM
Sodium phosphate buffer (pH 8):	50 mM
Calcium chloride:	10 mM
S9 homogenate:	100 µL/mL

4. Test cells: Mammalian cells in culture

- [ ] Mouse lymphoma L5178Y cells
- [x] Chinese hamster ovary (CHO) cells
- [ ] V79 cells (Chinese hamster lung fibroblasts)
- [ ] list any others

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?		Yes	Not reported	No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

Media: Ham's F12 medium (F12FBS5-Hx) without hypoxanthine, supplemented with 5% fetal bovine serum (FBS) and contained 100 units penicillin/mL, 100 µg streptomycin/mL, and 2 mM L-glutamine/mL.

5.Locus examined

	X	**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		Ouabain
		Fluorodeoxyuridine (FdU)	10 µM	6-thioguanine (6-TG)		
		Trifluorothymidine				

6. Test compound concentrations used

Preliminary cytotoxicity

Concentrations of 0, 0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 2000 µg cyantraniliprole/mL were evaluated in duplicate in the presence and absence of S9 activation.

Mutagenesis assay

Concentrations of 0, 50, 100, 150, 250, and 500 µg cyantraniliprole/mL were evaluated in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

18-August-2010 to 16-September-2010

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and ten concentrations of test substance ranging from 0.5 to 2000 µg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenicity assay

Treatment:

Cells ($4x10^5$cells/mL) were exposed to test compound, solvent or positive controls for 5 hours, respectively (non-activated) and 3 hours (activated).

After washing, cells were cultured for 7-9 days (expression period) before cell selection.

After expression, cells were plated: $2x10^5$cells/mL (5 plates/culture) were reincubated for 7-10 days in selection medium to determine numbers of mutants and 200 cells/culture (3 plates/culture) were reincubated for 7-10 days (3 plates/culture) without selective agent to determine the CE.

4. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies (MFs) with at least two consecutive doses showing MFs of >40 mutants per 10^6 clonable cells. The performing laboratory provided historical control data.

5. Statistics

The data did not warrant statistical analysis.

II.RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of 200 mg/mL. Cyantraniliprole was present at acceptable concentrations in the in the dosing solutions, which were found to range from 92.9% to 99.8% of nominal concentrations. Cyantraniliprole was shown to be stable in the dosing solutions under the conditions of the study. Cyantraniliprole was not found in the 0 mg/mL samples.

B. PRELIMINARY CYTOTOXICITY ASSAY

Visible precipitate in the treatment medium was apparent at test substance concentrations ≥500 µg/mL at the beginning and end of treatment. No visible precipitate was observed at concentrations ≤150 µg/mL. The CE at 2000 µg/mL was 51% without activation and 66% with activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 50 to 500 µg/mL for both the non-activated and S9-activated cultures.

C. MUTAGENESIS ASSAY

Summarized results are presented in Study Report Table 3 (nonactivated assay) and Table 4 (S9-activated assay). As shown, there was visible precipitate in the treatment medium at test substance concentrations ≥250 µg/mL at the beginning of treatment and only at 500 µg/mL at the end of treatment. Relative CEs were 70% and 83% at 500 µg/mL, the highest dose tested, in the non-activated and S9-activated systems, respectively. None of the treated cultures exhibited MFs greater than 40 mutants per 10^6 clonable cells. By contrast, the non-activated and S9-activated positive controls induced MFs that were at least 3 times that of the solvent control and exceeded 40 mutants per 10^6 clonable cells.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 476 (1997)

C. CONCLUSIONS: Cyantraniliprole was tested up to an insoluble concentration (500 µg/mL) but failed to induce a mutagenic response CHO cells in either the absence or the presence of S9 activation. The sensitivity of the test system to detect a mutagenic response was adequately demonstrated by the nonactivated and S-9-activated positive controls. Accordingly, it was concluded that cyantraniliprole was negative in this test system. **The assay was well conducted and acceptable for regulatory purposes.**

Deficiencies: None

TABLE 3

Non-activated (-S9) Study using cyantraniliprole

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates: Plate Counts 1	2	3	Average Colonies	Cloning Efficiency	Selection (Mutation) Plates: Plate Counts 1	2	3	4	5	Average Colonies	Mutants/10^6 Clonable Cells	Relative Cloning Efficiency (%)
Solvent	A		188	193	189	167.5	0.84	0	0	1	0	2	1.0	6.0	**100**
	B		158	136	141			0	0	2	2	3			
EMS (0.2µL/mL)	A		206	158	170	164.7	0.82	38	71	64	57	62	74.6	453.0	**69**
	B		131	138	185			87	83	87	96	101			
50	A		160	145	117	150.0	0.75	0	0	0	0	0	0.6	4.0	**84**
50	B		149	161	168			2	0	0	2	2			
100	A		159	171	150	164.8	0.82	0	0	0	0	0	0.4	2.4	**73**
100	B		183	172	154			1	0	1	0	2			
150	A		185	181	142	162.0	0.81	0	0	1	0	0	0.9	5.6	**85**
150	B		158	153	153			2	1	1	3	1			
250	A		173	147	187	155.0	0.78	0	0	0	0	1	0.5	3.2	**89**
250	B		122	149	152			0	0	1	2	1			
500	A	P	145	183	135	147.7	0.74	0	0	1	1	3	0.7	4.7	**70**
500	B	P	143	171	109			0	0	1	1	0			

Solvent = DMSO
A and B are duplicate cultures

P – Precipitating concentration

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{200 cells/dish}} \times 100$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

(Relative Cloning Efficiency copied from Table 2)

TABLE 4

Activated (+S9) Study using cyantraniliprole

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates: Plate Counts 1	2	3	Average Colonies	Cloning Efficiency	Selection (Mutation) Plates: Plate Counts 1	2	3	4	5	Average Colonies	Mutants/10^6 Clonable Cells	Relative Cloning Efficiency (%)
Solvent	A		163	153	135	158.8	0.79	0	2	0	0	2	0.6	3.8	**100**
	B		172	166	164			1	0	1	0	0			
B(a)P (4µg/mL)	A		149	163	132	137.7	0.69	16	18	11	21	24	17.2	124.9	**69**
	B		139	116	127			25	17	12	13	15			
50	A		171	171	161	158.3	0.79	0	0	0	1	0	0.4	2.5	**84**
50	B		166	138	143			0	0	1	2	0			
100	A		143	155	115	143.2	0.72	0	0	1	0	3	0.4	2.8	**73**
100	B		163	166	117			0	0	0	0	0			
150	A		127	136	110	124.3	0.62	0	0	1	0	0	0.6	4.8	**85**
150	B		***	***	***			0	0	1	4	0			
250	A		183	153	156	164.0	0.82	0	1	2	0	1	0.6	3.7	**89**
250	B		***	***	***			1	0	1	0	0			
500	A	P	123	153	117	144.8	0.72	0	0	0	1	2	0.5	3.5	**70**
500	B	P	159	156	161			1	0	0	1	0			

Solvent = DMSO
A and B are duplicate cultures

P – Precipitating concentration
*** - Culture lost to a plating error

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{200 cells/dish}} \times 100$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

(Relative Cloning Efficiency copied from Table 2)

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.1	***In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay**
Report:	Wagner, V.O. VanDyke, M.R. (2009b); Cyantraniliprole (DPX-HGW86) technical: Bacterial reverse mutation assay, DuPont-27900. BioReliance, Rockville, Maryland, USA **Report No.:** AC25SL.503.BTL. MRID 48208424
Dates of work:	03-March-2009 to 31-March-2009
Guidelines:	**Guidelines:** OPPTS 870.5100 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: NO (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In independent trials of a reverse gene mutation assay (MRID 48208424), cyantraniliprole (97%, Lot No. DXP-HGW86-412) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) in two phases using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The dose levels tested were 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the lack of mutagenic potential of the test substance. The dose levels tested were 0, 50, 150, 500, 1500, and 5000 µg/plate. The highest dose level was set based on insolubility of the test substance and compatibility with the target cells. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a maximum concentration of approximately 150 mg/mL.

Compound precipitation was observed at 5000µg per plate +/-S9 in both trials. The positive and vehicle controls fulfilled the requirements for a valid test. No positive mutagenic response or appreciable toxicity was observed in either the initial toxicity-mutation assay or the confirmatory mutagenicity assay.

Under the conditions of this study, cyantraniliprole was tested up to the limit dose and was negative for mutagenic activity in non-activated and S9-activated test systems.

The study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: HGW86-412
   Purity: 97.0%
   Description: Off-white fine powder
   CAS # 736994-63-1
   Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.) Dosing formulations were adjusted to 100% purity using a correction factor of 1.031.

2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)/50 µL

Positive control, non activation:

Positive control	Dose	Strain(s)
2-Nitrofluorene	1 µg/plate	TA98
Sodium azide	1 µg/plate	TA100, TA1535
9-aminoacridine	75 µg/plate	TA1537
methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A

Positive control, activation:

Positive control	Dose	Strain
2-Aminoanthracene	1 µg/plate	TA98, TA1535, TA1537
	2 µg/plate	TA100
	10 µg/plate	WP2 *uvr*A

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2359
   Source: Moltox, Inc., Boone, NC
   Protein content: 35.4 mg/mL
   Source: Not applicable
   Characterisation: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition:
   Phosphate buffer (pH 7.4): 100 mM
   Glucose-6-phosphate: 5 mM
   NADP: 4 mM
   KCl: 33 mM
   $MgCl_{2:}$ 8 mM
   S9: 10% (v/v)

4. Test organisms

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test concentrations for plate incorporation assay

Exp. No. B1: (Initial Toxicity-Mutation Assay)	Concentrations of 0, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg cyantraniliprole/plate were evaluated in duplicate in the presence and absence of S9 activation.
Exp. No. B2: (Confirmatory Mutagenicity Assay)	Concentrations of 0, 50, 150, 500, 1500 and 5000 µg cyantraniliprole/ plate were evaluated in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

03-March-2009 to 31-March-2009

2. Plate incorporation assay

This study consisted of 2 independent trials, Experiment No. B1 and Experiment No. B2. Experiment No. B1, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test substance were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537, and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. Experiment No. B2, the confirmatory mutagenicity test, was used to evaluate the mutagenic potential of the test substance. Five dose levels of test substance along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537, and WP2 *uvr*A on selective agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test substance, vehicle control and positive controls were plated in triplicate. One-half milliliter (0.5mL) of S9 or Sham mix, 100 µL of tester strain (containing approximately 0.3×10^9 bacteria), and 50 µL of vehicle or test substance dilution were added to 2 mL of molten selective top agar (0.8% [w/v] agar and 0.5% NaCl [w/v]) supplemented with L-histidine, D-biotin, and L-tryptophan) at 45 ± 2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37 ± 2°C. Plates that were not counted immediately following the incubation period were stored at 2 to 8°C prior to evaluation and counting of revertant colonies.

The condition of the bacterial background lawn was evaluated for evidence of test substance toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity was scored relative to the concurrent vehicle control plates and recorded with the mean revertant count for the strain, condition and concentration. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the assay was the preliminary toxicity assay or the plate exhibited toxicity.

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

For the test substance to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test substance. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100, and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal. The performing laboratory provided historical control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on the solubility of the test substance and compatibility with the target cells. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (90.9, 87.9, and 94.7% of their respective targets). The stability analysis of the formulations found the test substance to be stable in DMSO at room temperature for the period of dosing. No test substance was detected in the vehicle control sample.

B. MUTATION ASSAYS

Compound precipitation was observed in both trials at 5000 µg per plate. No appreciable toxicity was observed in either trial as is evidenced by a reduction of the microcolony background lawns and/or by a concentration-related decrease in mean revertants per plate. All positive controls exhibited more than a 3-fold increase in mean revertants over the respective mean of the vehicle controls. For all strains except TA1535 and TA1537, no test substance concentration produced a mean 2 times greater than the mean of its respective vehicle control. For strains TA1535 and TA1537, no test substance concentration produced a mean 3 times greater than the mean of its respective vehicle control. There was no concentration-related increase in the mean revertants per plate in any strain (Tables 1 and 2).

Controls: The positive and vehicle controls fulfilled the requirements for a valid test.

Table 1
Summary of average revertants/plate without activation of Cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2
Cyantraniliprole	0	18[a]	11[b]	146	123	9	14	4	5	27	28
	1.5	16	ne	151	ne	16	ne	7	ne	35	ne
	5.0	16	ne	158	ne	11	ne	7	ne	36	ne
	15	20	ne	85	ne	11	ne	5	ne	27	ne
	50	15	13	159	128	11	10	6	6	42	33
	150	15	15	150	117	12	13	5	6	27	26
	500	17	10	129	132	14	10	5	6	30	26
	1500	18	11	132	124	8	11	4	4	35	24
	5000	19	13	141	128	16	13	5	6	34	29
2NF	1	179	200	ne	ne	ne	ne	ne	ne	ne	ne
NAAZ	1	ne	ne	567	384	447	280	ne	ne	ne	ne
9AA	75	ne	ne	ne	ne	ne	ne	2186	1521	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	458	364

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

2NF = 2-nitrofluorene; NAAZ = Sodium azide; 9AA = 9-aminoacridine; MMS = Methyl methanesulfonate;

ne = Not evaluated

Data were derived from Tables 21 and 22, pp. 42 and 43, MRID 48208424.

Table 2
Summary of average revertants/plate with activation of Cyantraniliprole

Compound	Conc. µg/plate	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
		Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2	Exp. B1	Exp. B2
Cyantraniliprole	0	28[a]	19	155	107	10	12	6	9	38	35
	1.5	25	ne	153	ne	10	ne	4	ne	37	ne
	5.0	30	ne	140	ne	10	ne	6	ne	39	ne
	15	24	ne	133	ne	15	ne	8	ne	40	ne
	50	22	21	139	119	13	13	7	4	40	34
	150	24	21	147	140	11	12	5	7	38	39
	500	22	16	140	114	15	13	5	7	33	33
	1500	21	17	146	107	16	13	6	5	42	31
	5000	24	18	143	118	13	9	8	8	31	30
2AA	1	438	407	ne	ne	86	61	45	62	ne	ne
2AA	2	ne	ne	944	527	ne	ne	ne	ne	ne	ne
2AA	10	ne	ne	ne	ne	ne	ne	ne	ne	170	280

[a] Average of 2 replicates per trial; B1 = Initial Toxicity-Mutation Assay

[b] Average of 3 replicates per trial; B2 = Confirmatory Mutagenicity Assay

2AA-1 = 2-aminoanthracene

ne = not evaluated

Data were derived from Tables 21 and 22, pp. 42 and 43, MRID 48208424.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 471(1997)

C. CONCLUSIONS: Cyantraniliprole was negative for mutagenic activity up to precipitating concentrations in all strains in both trials in the presence and absence of S9-activation in the *in vitro* bacterial gene mutation assay. There were no treatment-related increases in the mean number of revertants/plate in any strain (+/-S9). The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

Accordingly, Cyantraniliprole is negative in this test system in a well-done study.

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Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.2/02 ***In vitro* Mammalian Chromosome Aberration Test**

Report:	Gudi, R., Rao, M. (2009); Cyantraniliprole (DPX-HGW86) technical: *In vitro* mammalian chromosome aberration test. DuPont-27559; BioReliance, Rockville, Maryland, USA,Report No.: AC25MV.341.BTL (MRID 48208425).
Dates of work:	26-February-2009 to 23-March-2009
Guidelines:	OPPTS 870.5375 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: Yes (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.
Exceptions	No

Executive Summary:

Cyantraniliprole (97.7%; Lot No. 9182-3b) was tested in the *in vitro* mammalian chromosome aberration test (MRID 48208425) using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). The test substance was soluble in dimethyl sulfoxide (DMSO) at a maximum concentration of approximately 200 mg/mL in the solubility test. The test substance formed a workable suspension at 335 mg/mL and concentrations ≤100.5 mg/mL were soluble in DMSO in the preliminary toxicity assay and was soluble in DMSO at all concentrations tested in the chromosome aberration assay. In the preliminary toxicity assay, the cells were exposed to 9 concentrations of the test substance ranging from 0.335 to 3350 µg/mL, as well as a vehicle control. Visible precipitate was observed in treatment medium at ≥1005 µg/mL and levels ≤335 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Substantial toxicity (i.e., ≥50% reduction in the mitotic index, MI) was observed at ≥1005 µg/mL in all three-exposure groups. Based on these findings, doses chosen for the chromosome aberration assay were 0, 62.5, 125, 250, 500, 600, 700, 800, and 900 µg/mL for the 4-hour non-activated and activated treatment conditions, and 0, 31.3, 62.5, 125, 250, 400, 500, 600, 700, and 800 µg/mL for the 20-hour non-activated treatment condition. HPBL were treated for 4 hours (activated test system), and 4 and 20 hours (non-activated test system). After exposure to Colcemid®, metaphase cells were harvested at approximately 20 hours from the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition) then structural and numerical chromosome aberrations.

In the chromosome aberration assay, visible precipitate was observed in treatment medium at ≥ 500 µg/mL and levels ≤ 400 µg/mL were soluble in treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, the non-activated 4-hour exposure group, visible precipitate was observed in treatment medium at ≥ 700 µg/mL and concentrations ≤ 600 µg/mL were soluble in treatment medium. In the S9-activated 4-hour and non-activated 20-hour exposure groups, visible precipitate was observed at ≥ 600 µg/mL and doses ≤ 500 µg/mL were soluble in treatment medium at the conclusion of the treatment period.

Toxicity (mitotic inhibition) in excess of 50%, relative to the solvent control, was observed at concentrations ≥800 µg/mL in the non-activated 4-hour exposure group, ≥700 µg/mL in the S9-activated 4-h exposure group, and at concentrations ≥500 µg/mL in the non-activated 20-h exposure group. Positive controls induced the appropriate response. No statistically significant increases in structural chromosome aberrations were observed at any of the concentrations evaluated ($p > 0.05$, Fisher's Exact test) in either the presence or absence of metabolic activation.

Based on the findings of this study, cyantraniliprole was concluded to be negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes with and without an exogenous metabolic activation system.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for an i*n vitro* mammalian cytogenetics (chromosome aberrations) assay (OPPTS 870.5375; OECD 473).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test material:	Cyantraniliprole technical
Lot/Batch #:	9182-3B
Purity:	97.7%
Description:	Pale yellow powder
CAS #	736994-63-1
Stability of test compound:	Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study. Dose formulations were adjusted to 100% purity using a correction factor of 1.044.

2. Control materials

Solvent control:	Dimethyl sulfoxide (DMSO)
Positive, non-activation:	Mitomycin C (MMC) in water at 0.3 and 0.6 µg/mL
Positive, activation:	Cyclophosphamide (CP) in water at 20 µg/mL

3. Activation:

Activation:	Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
Lot number:	2278
Source:	Moltox, Inc., Boone, NC
Protein content:	Not provided
Characterisation:	The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

S9 mix composition

Glucose-6-phosphate:	1 mM
NADP:	1 mM
KCl:	6 mM
$MgCl_2$:	2 mM
S9:	20 µL per mL medium

4. Test cells

Human lymphocytes were obtained from the peripheral blood of a healthy, non-smoking adult female and were cultured in RPMI 1640 supplemented with 1% phytohemagglutinin (PHA). Cultures were incubated for 44-48 hours prior to testing.

5. Culture medium (for the assays)

RPMI 1640, serum-free medium containing 15% fetal bovine serum, antibiotics and 2 mM L-glutamine

6. Test compound concentrations evaluated

Preliminary cytotoxicity test: 0, 0.335, 1.005, 3.35, 10.05, 33.5, 100.5, 335, 1005, and 3350 µg cyantraniliprole/mL +/-S9.

Chromosome aberration assay:

Non-activated 4-hour exposure group	0, 125, 250, and 800 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.
S9-activated 4-hour exposure group	0, 250, 500, and 700 µg cyantraniliprole/mL in duplicate in the presence of S9 activation.
Non-activated 20-hour exposure group	0, 62.5, 125, and 500 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

26-February-2009 to 23-March-2009

2. Treatment:

Preliminary cytotoxicity assay: The toxicity test was performed to select concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, the percentage of cells in mitosis per 500 cells counted). The cells were exposed to solvent alone and to nine concentrations of the test substance for 4 hours in both the presence and absence of S9 activation, and for 20 hours continuously in the absence of S9 activation.

Cytogenetic assay: Independently performed experiments were conducted

a. Cell exposure time:

	Test material	Solvent control	Positive control
Non-activated:	4 h	4 h	4 h
Activated:	20 h	20 h	20 h
	4 h	4 h	4 h

b. Spindle inhibition:

Inhibition used/concentration:	Colcemid/ 0.1 µg/mL
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

	Test material	Solvent control	Positive control
Non-activated and Activated:	16 or 0 h & 16 h	16 or 0 h & 16 h	16 or 0 h & 16 h

d. Details of slide preparation: Following the 2-hour colcemid treatment, cells were treated with hypotonic KCl (0.075 M) for 20 min at 37° C. Cells were then fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5 % Giemsa. Two slides per group per experiment were prepared.

e. Metaphase analysis

No. of cells examined per dose: 200 (100 per duplicate culture) were scored.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy cells		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically

elevated relative to the concurrent negative control group ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historical solvent controls may be judged as not biologically significant. The performing laboratory provided historical control data.

g. Statistical analysis: Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose-responsiveness.

II. RESULTS AND DISCUSSION

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of approximately 200 mg/mL. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (within 6.1% of nominal concentrations). Cyantraniliprole was also shown to be stable in the dosing solutions under the conditions of the study. Cyantraniliprole was not found in the 0 mg/mL samples. The test material at the lowest precipitating dose (1005 µg/mL) had no adverse effect on the pH or osmotic pressure of the culture medium.

B. PRELIMINARY CYTOTOXICITY ASSAY

Visible precipitate was observed in treatment medium at ≥1005 µg/mL and doses ≤335 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Substantial toxicity (≥50%) was observed at dose levels ≥1005 µg/mL in all three exposure groups. Based on these results, concentrations presented below in Table 1 were selected for the chromosome aberration assay:

Table 1. Dose selection for the chromosome aberrations assay with cyantraniliprole

Treatment condition	Treatment time (h)	Concentrations (µg/mL)
Non-activated	4	0, 62.5, 125, 250, 500, 600, 700, 800, 900
	20	0, 31.3, 62.5, 125, 250, 400, 500, 600, 700, 800
S9 activated	4	0, 62.5, 125, 250, 500, 600, 700, 800, 900

C. CHROMOSOME ABERRATION ASSAY

In the chromosome aberration assay, visible precipitate was observed in treatment medium at ≥ 500 µg/mL and ≤ 400 µg/mL were soluble in treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, the non-activated 4-hour exposure group, visible precipitate was observed in treatment medium at levels of ≥ 700 µg/mL and levels ≤ 600 µg/mL were soluble in treatment medium. In the S9-activated 4-hour and non-activated 20-hour exposure groups, visible precipitate was observed at ≥ 600 µg/mL and levels ≤ 500 µg/mL were soluble in treatment medium at the conclusion of the treatment period. Summarized results from the chromosome aberration assays are presented in Study Report Table 7 and are as follows:

Non-activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 800 µg/mL, mitotic inhibition was 56%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated group was not increased relative to the solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

Activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 700 µg/mL, mitotic inhibition was 52%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated group was not increased relative to the solvent control at any dose level (p >0.05, Fisher's Exact test).

Non-activated 20 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 500 µg/mL, mitotic inhibition was 53%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated groups was not increased relative to the solvent control at any dose level (p >0.05, Fisher's Exact test).

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable (EPA rating Acceptable/guideline)

This study is fully compliant with OECD 473 (1997)

C. CONCLUSIONS: Cyantraniliprole was tested up to adequate insoluble concentrations [400-500 µg/mL–S9 (4- and 20-hour treatments) and 700 µg/mL +S9 (4-hour treatment)] but failed to induce a clastogenic effect. The expected responses were obtained with the negative and positive controls either with or without S9 activation at both time intervals. Based on these considerations, it was, therefore, concluded that cyantraniliprole was negative in this test system in a well-conducted study.

Deficiencies: None

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	8.4	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
125	-S9	4	9.0	200	200	0.000	±0.000	0.0	0.0
250	-S9	4	8.3	200	200	0.000	±0.000	0.0	0.0
800	-S9	4	3.7	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	4.3	200	100	0.250	±0.657	0.0	17.0**
DMSO	+S9	4	6.9	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
250	+S9	4	7.2	200	200	0.000	±0.000	0.0	0.0
500	+S9	4	5.9	200	200	0.000	±0.000	0.0	0.0
700	+S9	4	3.3	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	2.0	200	100	0.200	±0.512	0.0	15.0**
DMSO	-S9	20	8.3	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
62.5	-S9	20	7.0	200	200	0.000	±0.000	0.0	0.0
125	-S9	20	7.0	200	200	0.000	±0.000	0.0	0.0
500	-S9	20	3.9	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	3.5	200	100	0.190	±0.465	0.0	16.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.2/01 ***In vitro* Mammalian Chromosome Aberration Test**

Report:	Gudi, R., Rao, M. (2009); Cyantraniliprole (DPX-HGW86) technical: *In vitro* mammalian chromosome aberration test. DuPont-27901; BioReliance, Rockville, Maryland, USA,Report No.: AC25SL.341.BTL (MRID 48208426).
Dates of work:	26-February-2009 to 20-March-2009
Guidelines:	OPPTS 870.5375 (1998), ECC 2000/32/EC, Annex 4D-B13/14 No., L136 (2000), OECD No. 471 (1998), JMAFF 12 Nousan 8147 Guideline No.2-1-19-1 (2000 and later revisions). **Deviations:** None
GLP: NO (certified laboratory)	Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.
Exceptions	No

Executive Summary:

Cyantraniliprole (97.0%; Lot No. HGW86) was tested in the *in vitro* mammalian chromosome aberration test (MRID 48208426) using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). In the preliminary toxicity assay, the cells were exposed to 9 concentrations of the test substance ranging from 0.431 to 4310 µg/mL, as well as a vehicle control. The highest dose level was set based on production of cytotoxicity in a preliminary toxicity assay and insolubility of the test substance at higher doses in a preliminary toxicity assay. Visible precipitate was observed in treatment medium at ≥1293 µg/mL and levels ≤431 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Substantial toxicity (≥50% reduction in the mitotic index, MI) was observed at 4310 µg/mL in the non-activated and S9-activated 4-hour exposure groups and at concentrations ≤431 µg/mL in the non-activated 20-hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 125 to 3500 µg/mL for the non-activated and S9-activated 4-hour exposure groups, and from 15.7 to 1500 µg/mL for the non-activated 20-hour exposure group. Cells were evaluated for toxicity and structural and numerical chromosome aberrations.

Compound precipitation was recorded at ≥1000µg/mL. MIs were reduced by 21 or 15% at 1000 µg/mL without or with S9 activation, respectively (4-hour treatment) or by 54% at 500 µg/mL –S9 (20-hour treatment). However, no statistically significant increases in structural chromosome aberrations were observed at any of the concentrations evaluated ($p > 0.05$, Fisher's Exact test) in either the presence or absence of metabolic activation. Positive controls induced the appropriate response.

Based on the findings of this study, cyantraniliprole was concluded to be negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes with and without an exogenous metabolic activation system.

This study is classified as **totally reliable (acceptable/guideline)** and satisfies the guideline requirement for an i*n vitro* mammalian cytogenetics (chromosome aberrations) assay (OPPTS 870.5375; OECD 473).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyantraniliprole technical
   Lot/Batch #: HGW86-412
   Purity: 97.0%
   Description: Solid powder
   CAS # 736994-63-1
   Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study. Dose formulations were adjusted to 100% purity using a correction factor of 1.031.

2. Control materials
   Solvent control: Dimethyl sulfoxide (DMSO)
   Positive, non-activation: Mitomycin C (MMC) in water at 0.3 and 0.6 µg/mL
   Positive, activation: Cyclophosphamide (CP) in water at 20 µg/mL

3. Activation: Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
   Lot number: 2278
   Source: Moltox, Inc., Boone, NC
   Protein content: Not provided
   Characterisation: The metabolic activation ability of the S9 was assayed for its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100.

   S9 mix composition
   Glucose-6-phosphate: 1 mM
   NADP: 1 mM
   KCl: 6 mM
   $MgCl_{2:}$ 2 mM
   S9: 20 µL per mL medium

4. Test cells

   Human lymphocytes were obtained from the peripheral blood of a healthy, non-smoking adult female and were cultured in RPMI 1640 supplemented with 1% phytohemagglutinin (PHA). Cultures were incubated for 44-48 hours prior to testing.

5. Culture medium (for the assays)

   RPMI 1640, serum-free medium containing 15% fetal bovine serum, antibiotics and 2 mM L-glutamine

6. Test compound concentrations evaluated:

   Preliminary cytotoxicity test: 0, 0.431, 1.293, 4.31, 12.93, 43.1, 129.3, 431, 1293, 4310 µg cyantraniliprole/mL +/-S9.

Chromosome aberration assay:

Non-activated 4-hour exposure group	0, 250, 500, and 1000 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.
S9-activated 4-hour exposure group	0, 250, 500, and 1000 µg cyantraniliprole/mL in duplicate in the presence of S9 activation.
Non-activated 20-hour exposure group	0, 125, 250, and 500 µg cyantraniliprole/mL in duplicate in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

26-February-2009 to 20-March-2009

2. Treatment:

Preliminary cytotoxicity assay: The toxicity test was performed to select concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, the percentage of cells in mitosis per 500 cells counted). The cells were exposed to solvent alone and to nine concentrations of the test substance for 4 hours in both the presence and absence of S9 activation, and for 20 hours continuously in the absence of S9 activation.

Cytogenetic assay: Independently performed experiments were conducted

a. Cell exposure time:

	Test material	Solvent control	Positive control
Non-activated:	4 h 20 h	4 h 20 h	4 h 20 h
Activated:	4 h	4 h	4 h

b. Spindle inhibition:

Inhibition used/concentration:	Colcemid/ 0.1 µg/mL
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

	Test material	Solvent control	Positive control
Non-activated and Activated:	16 or 0 h & 16 h	16 or 0 h & 16 h	16 or 0 h & 16 h

d. Details of slide preparation: Following the 2-hour colcemid treatment, cells were treated with hypotonic KCl (0.075 M) for 20 min at 37° C. Cells were then fixed with a 3:1mixture of methanol and glacial acetic acid, and stained with 5 % Giemsa. Two slides per group per experiment were prepared.

e. Metaphase analysis

No. of cells examined per dose: 200 (100 per duplicate culture) were scored.

Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes, polyploidy cells		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent negative control group ($p \leq 0.05$). However, values that are statistically

significant but do not exceed the range of historical solvent controls may be judged as not biologically significant. The performing laboratory provided historical control data.

g. Statistical analysis: Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose-responsiveness.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The Sponsor conducted the test substance dose formulation analysis. The results of the homogeneity analysis indicate that the mean of the top, middle, and bottom portions of the analysed sample (350 mg/mL) were 104.3%, 112.6%, and 114.6% of their respective nominal concentrations. The results of the concentration analysis indicate that the analysed samples (1.57 and 100 mg/mL) were 114.6% and 100.0% of their respective nominal concentrations. Cyantraniliprole was shown to be stable in the dosing solutions under the conditions of the study. Cyantraniliprole was not found in the 0 mg/mL samples. The test material at the lowest precipitating dose (1293 µg/mL) had no adverse effect on the pH or osmotic pressure of the culture medium.

B. PRELIMINARY CYTOTOXICITY ASSAY

Visible precipitate was observed in treatment medium at concentrations ≥1293 µg/mL and levels ≤431 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. At the 4-hour exposure, no cells survived treatment with 4310 µg/mL in the non-activated and S9-activated series. Severe toxicity (100% mitotic inhibition) was observed at 4310 µg/mL. For the non-activated, 20-hour exposure groups, MIs were ≤50% at levels ≥431 µg/mL. Based on these results, concentrations presented below in Table 1 were selected for the chromosome aberration assay:

Table 1. Dose selection for the chromosome aberrations assay with cyantraniliprole

Treatment condition	Treatment time (h)	Recovery time (h)	Concentrations (µg/mL)
Non-activated	4	16	0, 125, 250, 500, 1000, 2000, 2500, 3000, 3500
	20	0	0, 15.7, 31.3, 62.5, 125, 250, 500, 1000, 1500
S9 activated	4	16	0, 125, 250, 500, 1000, 2000, 2500, 3000, 3500

C. CHROMOSOME ABERRATION ASSAY

In the chromosome aberration assay, visible precipitate was observed in treatment medium at dose levels ≥ 1000 µg/mL and dose levels of ≤ 500 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. Summarized results from the chromosome aberration assays are presented in Study Report Table 7 and are as follows:

Non-activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 1000 µg/mL, mitotic inhibition was 21%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test).

Activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 1000 µg/mL, mitotic inhibition was 15%, relative to the solvent control. The percentage of cells with structural or

numerical aberrations in the test substance-treated groups was not increased relative to the solvent control at any dose level ($p > 0.05$, Fisher's exact test).

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	16.0	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
250	-S9	4	15.2	200	200	0.000	±0.000	0.0	0.0
500	-S9	4	16.1	200	200	0.000	±0.000	0.0	0.0
1000	-S9	4	12.7	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	13.9	200	100	0.220	±0.484	0.0	19.0**
DMSO	+S9	4	17.0	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
250	+S9	4	16.9	200	200	0.000	±0.000	0.0	0.0
500	+S9	4	16.0	200	200	0.000	±0.000	0.0	0.0
1000	+S9	4	14.4	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	4.2	200	100	0.230	±0.446	0.0	22.0**
DMSO	-S9	20	15.8	200	200	0.000	±0.000	0.0	0.0
cyantraniliprole									
125	-S9	20	16.1	200	200	0.000	±0.000	0.0	0.0
250	-S9	20	12.4	200	200	0.000	±0.000	0.0	0.0
500	-S9	20	7.2	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	9.3	200	100	0.230	±0.446	0.0	22.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using the Fisher's Exact test.

Non-activated 20 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 500 µg/mL, mitotic inhibition was 54%, relative to the solvent control. The percentage of cells with structural or numerical aberrations in the test substance-treated groups was not increased relative to the solvent control at any dose level ($p > 0.05$, Fisher's exact test).

Controls: The positive controls fulfilled the requirements for a valid test.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable (EPA rating Acceptable/guideline)

This study is fully compliant with OECD 473 (1997)

C. CONCLUSIONS: Cyantraniliprole was tested up to adequate insoluble concentrations [1000 µg/mL +/–S9 (4-hour treatments) and 500 µg/mL –S9 (20-hour treatment)] but failed to induce a clastogenic effect. The expected responses were obtained with the negative and positive controls either with or without S9 activation at both time intervals. Based on these considerations, it was, therefore, concluded that cyantraniliprole was negative in this test system in a well-conducted study.

Deficiencies: None

Revised by U.S.	EPA (Primary Reviewer: Nancy McCarroll)
Report: IIA 5.4.3/02	Stankowski, L.F. (2011); Cyantraniliprole (DPX-HGW86) technical: CHO/HPRT forward mutation assay with duplicate cultures. DuPont-31372 Covance Laboratories, Inc., Vienna, Virginia, USA Report No.: 8236883 (MRID 48208443)
Dates of work:	01-October-2010 to 19 November-2010
Guidelines:	OPPTS 5300 (1998) OECD 476 (1997) UKEMS Guidelines (1990) JMAFF 12-Nousan-8147 (2000) JMAFF, draft
GLP: Yes (certified laboratory)	OECD Principles of GLP, ENV/MC/CHEM (1998) Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

Cyantraniliprole (97%; Lot No. 9182-1) was tested in the CHO/HGPRT mutation assay (MRID 48208443) with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9). The preliminary toxicity assay was used to establish the dose range for the mutagenesis assay. Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K_1-BH_4 cells were exposed for 5 hours at 37 ± 1°C to the test substance at concentrations of 0, 10.0, 25.0, 50.0, 100, 250, 500, 750, and 1000 µg/mL, in both the absence and presence of S9. The highest dose level was set based on production of cytotoxicity in a range finding experiment. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-thioguanine (2-amino-6-mercaptopurine)-resistant phenotype. The test material precipitated from solution in the aqueous treatment medium at concentrations ≥433 µg/mL. Ethyl methanesulfonate (EMS) and methylcholanthrene (MCA) were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of ≤50% of the concurrent vehicle controls.

In the mutation assay, compound precipitation was observed at concentrations in treatment medium ≥250 µg/mL. Relative cloning efficiency was 29.57% and 45.89% at 1000 µg/mL, the highest dose tested, in the non-activated and S9-activated systems, respectively. The positive controls induced the expected mutagenic responses. There was, however, no statistically significant or dose-dependent increase in the average mutant frequency observed with or without S9.

It was concluded; therefore, that Cyantraniliprole was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.

This study is classified as **totally reliable** (**acceptable/guideline)** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole technical
	Lot/Batch #:	9182-1
	Purity:	97.0%
	Description:	Solid, powder
	CAS #:	736994-63-1
	Stability of test compound:	The test substance was at the targeted concentrations and stable when stored at room temperature during the dosing period in the vehicle for all dose levels. Dose formulations were adjusted to 100% purity using a correction factor of 1.03.
2.	Control materials	
	Solvent control:	Dimethylsulfoxide (DMSO)
	Positive, non-activation:	Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 200 µg/mL
	Positive, activation:	Methylcholanthrene (MCA) in DMSO at a final concentration of 5.00 µg/mL
3.	Activation:	Rat liver S9 from Male Sprague-Dawley rats (Aroclor 1254-induced)
	Lot number:	2502
	Source:	Molecular Toxicology, Inc., Boone, NC
	Protein content:	36.0 mg/mL
	Characterization:	The metabolic activation ability of the S9 was characterized using varying S9 and positive control concentrations.
	S9 mix composition (final concentration in cultures):	
	NADP:	0.8 mM
	Glucose-6-phosphate:	1.0 mM
	KCl:	6.0 mM
	$MgCl_2$:	2.0 mM
	Sodium phosphate buffer (pH 7.5):	10 mM
	Calcium chloride:	2.0 mM
	S9 homogenate:	10 µL/mL

4. Test cells: Mammalian cells in culture

[ ]	Mouse lymphoma L5178Y cells	[ ]	V79 cells (Chinese hamster lung fibroblasts)
[x]	Chinese hamster ovary (CHO) cells	[ ]	list any others

Properly maintained?	[X]	Yes	[ ]		No
Periodically checked for Mycoplasma contamination?	[X]	Yes	[ ]		No
Periodically checked for karyotype stability?	[ ]	Yes	[ ]	Not reported	No
Periodically "cleansed" against high spontaneous background?	[X]	Yes	[ ]		No

Media: Ham's F12 medium (F12FBS5-Hx) without hypoxanthine, supplemented with 5% fetal bovine serum (FBS) and contained 100 units penicillin/mL, 100 µg streptomycin/mL, and 2 mM L-glutamine/mL.

5.Locus examined

	X	**Thymidine kinase (TK)**		**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**		**Na^+/K^+ ATPase**
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		Ouabain
		Fluorodeoxyuridine (FdU)	10 µM	6-thioguanine (6-TG)		
		Trifluorothymidine				

6. Test compound concentrations used

Preliminary cytotoxicity assay:	Concentrations of 0, 3.79, 7.58, 15.2, 30.3, 60.6, 121, 243, 485, 970, and 1940 µg cyantraniliprole/mL were evaluated in duplicate in the presence and absence of S9 activation.
Mutagenesis assay:	Concentrations of 0, 10.0, 25.0, 50.0, 100, 250, 500, 750, and 1000 µg cyantraniliprole/mL were evaluated in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

01-October-2010 to 19-November-2010

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and ten concentrations of test substance ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenicity assay

a. Treatment:

Cells (4x10^5cells/mL) were exposed to test compound, solvent or positive controls for 5 hours, respectively (non-activated) and 3 hours (activated).

After washing, cells were cultured for 7 days (expression period) before cell selection.

After expression, cells were plated: 2x10^5cells/mL (12 plates/culture) were reincubated for 8 days in selection medium to determine numbers of mutants and 200 cells/culture (3 plates/culture) were reincubated for 8 days without selective agent to determine the CE.

b. Evaluation criteria

The assay was considered positive when there was a statistically significant increase in mutant frequency that was at least 15 mutants/10^6 clonable cells over the concurrent vehicle controls, a dose response was observed and the response was reproducible. The performing laboratory provided historical control data.

c. Statistics

Data were evaluated according to the method of Snee and Irr (1981), *i.e.*, mutation frequencies were transformed as follows: $Y=[X+1]^{0.15}$ and analyzed by Student's T-test at $p \leq 0.05$.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of 173 mg/mL. Cyantraniliprole was present at acceptable concentrations in the dosing solutions (91.6 and 87.5% of their respective targets). The stability analysis of the formulations found the test substance to be stable in DMSO at room temperature for the period of dosing. No test substance was detected in the vehicle control sample. Based on these results, concentrations of 3.79 to 1940 µg/mL were selected for the preliminary assessment.

B. PRELIMINARY CYTOTOXICITY ASSAY

Precipitate was observed at concentrations ≥243 µg/mL. Relative CEs were 44.52% and 27.87% at 243 µg/mL, the lowest precipitating dose tested, in the non-activated and S9-activated systems, respectively. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 10 to 1000 µg/mL for both the non-activated and S9-activated cultures.

C. MUTAGENESIS ASSAY

Summarized results of the mutation assay are presented in Study Report Tables 2 and 3. As shown, precipitate was observed at concentrations in the treatment medium ≥250 µg/mL. Relative CE was 45.89% and 29.57% at 1000 µg/mL, the highest dose tested, in the non-activated and S9-activated systems, respectively. There was no statistically significant or dose-dependent increase in the average mutant frequency observed with or without S9.

Controls: The non-activated and S9-activated positive controls induced large increases in mutant frequencies and met the minimum criteria for a positive response.

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 476 (1997)

C. CONCLUSIONS: Cyantraniliprole was tested up to insoluble and/or cytotoxic concentrations but failed to induce a mutagenic response in CHO cells in either the absence or the presence of S9 activation. The sensitivity of the test system to detect a mutagenic response was adequately demonstrated by the nonactivated and S-9-activated positive controls. Accordingly, it was concluded that cyantraniliprole was negative in this test system. **The assay was well conducted and acceptable for regulatory purposes.**

Deficiencies: None

Table 2. Mutation Assay with S9

Study No.: 8236883
Test Article: Cyantraniliprole
Treatment Date: 02 November 2010

Treatment (+S9)	Adjusted Relative Survival (%)[a]	Average Adjusted Relative Survival (%)	Total Mutant Colonies	Cloning Efficiency (%)[b]	TG^r Mutants/ 10^6 Clonable Cells[c]	Average TG^r Mutants/ 10^6 Clonable Cells
DMSO (%, v/v)						
1.00	101.17	100.00	22	116.00	7.9	4.5
1.00	98.83		3	118.00	1.1	
Cyantraniliprole (µg/mL)						
10.0	110.96	101.96	-	-	-	-
10.0	92.96		-	-	-	
25.0	82.92	88.94	-	-	-	-
25.0	94.95		-	-	-	
50.0	127.64	110.79	8	107.33	3.1	2.7
50.0	93.94		6	107.50	2.3	
100	67.11	70.71	14	115.17	5.1	11.1
100	74.31		38	92.67	17.1	
250[d]	54.86	70.83	-	-	-	-
250[d]	86.80		-	-	-	
500[d]	43.79	43.25	12	79.00	6.3	5.9
500[d]	42.72		12	91.00	5.5	
750[d]	52.03	45.09	29	90.17	13.4	11.1
750[d]	38.14		18	86.00	8.7	
1000[d]	43.54	45.89	12	101.17	4.9	6.5
1000[d]	48.23		16	83.67	8.0	
MCA (µg/mL)						
5.00	39.67	35.56	251	96.83	108.0	129.8**
5.00	31.46		313	86.00	151.6	

a Day 1 cell count x cloning efficiency, as compared to the average of the concurrent vehicle controls (average cloning efficiency = 85.83%)
b Number of colonies / number of cells plated x 100% (average of three plates; average cloning efficiency = 117%)
c Total mutant colonies / number of cells selected, corrected for cloning efficiency (at time of selection)
d Test article incompletely soluble at end of treatment.
** Significant increase ($p \leq 0.01$); Snee and Irr, 1981.

Table 3. Mutation Assay without S9

Study No.: 8236883
Test Article: Cyantraniliprole
Treatment Date: 02 November 2010

Treatment (-S9)	Adjusted Relative Survival (%)[a]	Average Adjusted Relative Survival (%)	Total Mutant Colonies	Cloning Efficiency (%)[b]	TG^r Mutants/ 10^6 Clonable Cells[c]	Average TG^r Mutants/ 10^6 Clonable Cells
DMSO (%, v/v)						
1.00	83.57	100.00	21	111.50	7.8	6.2
1.00	116.43		9	83.33	4.5	
Cyantraniliprole (µg/mL)						
10.0	118.34	114.53	-	-	-	-
10.0	110.72		-	-	-	
25.0	104.58	92.38	-	-	-	-
25.0	80.19		-	-	-	
50.0	99.20	104.61	24	77.00	13.0	9.2
50.0	110.01		11	83.83	5.5	
100	61.57	62.51	13	74.83	7.2	6.9
100	63.46		14	88.33	6.6	
250[d]	48.85	50.02	-	-	-	-
250[d]	51.20		-	-	-	
500[d]	33.48	36.82	15	78.83	7.9	10.1
500[d]	40.15		23	77.67	12.3	
750[d]	38.42	37.71	8	89.17	3.7	9.5
750[d]	37.00		32	87.67	15.2	
1000[d]	29.94	29.57	18	82.67	9.1	6.5
1000[d]	29.21		8	86.33	3.9	
EMS (µg/mL)						
200	46.18	43.48	299	81.00	153.8	151.5
200	40.78		286	79.83	149.3	

a Day 1 cell count x cloning efficiency, as compared to the average of the concurrent vehicle controls (average cloning efficiency = 70.8%)
b Number of colonies / number of cells plated x 100% (average of three plates; average cloning efficiency = 97.42%)
c Total mutant colonies / number of cells selected, corrected for cloning efficiency (at time of selection)
d Test article incompletely soluble at end of treatment.
** Significant increase ($p \leq 0.01$); Snee and Irr, 1981.

Revised by U.S. Environmental Protection Agency (Primary Reviewer: Nancy McCarroll)

IIA 5.4.4/01 *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

Report: Donner, E.M. (2011); Cyantraniliprole (DPX-HGW86) technical: Mouse bone marrow micronucleus test. DuPont Haskell Laboratories, Newark, DE;DuPont-31373. (MRID 48208444).

Dates of work: 04-October-2010 to 02-December-2010

Guidelines: OPPTS 870. 5395 (1998)
OECD 474 (1997)
UKEMS Guidelines (1990)
JMAFF 12-Nousan-8147 (2000)
JMAFF, draft

GLP: Yes OECD Principles of GLP, ENV/MC/CHEM (1998)
Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Exceptions: No

Executive Summary:

Cyantraniliprole technical (97%; Lot No. 9182) was evaluated in the mouse micronucleus assay (MRID 48208444) for its ability to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) in male and female Crl:CD1(ICR) mice. Doses were 0, 500, 1000 or 2000 mg/kg by single-dose oral gavage, based on the range finding results. Concurrent control groups were administered 0.1% Tween-80 in aqueous methylcellulose (0.5%), the vehicle (negative) control, or 40 mg/kg of cyclophosphamide (positive control). The vehicle control and the low- and intermediate-dose groups contained 10 animals/sex. The high-dose group contained 14 animals/sex. The positive control group consisted of 5 animals/sex. Half of the animals in each test substance and vehicle control group were sacrificed at each time point, approximately 24 or 48 hours post-dosing, respectively. The positive control group was sacrificed approximately 24 hours post-dosing. Bone marrow smears were prepared immediately after the sacrifices. In-life observations included clinical signs and body weight determinations. Two thousand PCEs per animal were evaluated for micronuclei and 1000 total erythrocytes per animal were evaluated for bone marrow toxicity.

In the main study, there were no clinical signs of toxicity or deaths and significant changes in body weight or body weight gain was not seen in either male or female animals administered the test substance. Similarly, there was not evidence of a cytotoxic effect on the bone marrow cells. The positive and vehicle controls induced the expected response. No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance-treated group of male or female animals at the 24-hour time point or in female animals at the highest dose level at the 48-hour time point. A statistically significant increase, which was observed at the highest dose in male animals at the 48-hour time point, was considered spurious, since it was within laboratory historical control range, and without biological relevance.

Under the conditions of this study, cyantraniliprole was neither clastogenic nor aneugenic in the mouse bone marrow micronucleus assay.

This study is classified as **totally reliable** (**acceptable/guideline)** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic -erythrocyte micronucleus assay in mice mutagenicity data.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole technical
	Lot/Batch #:	9182
	Purity:	97.0%
	Description:	Off-white solid
	CAS #:	736994-63-1
	Stability of test compound:	Homogeneity and target concentrations were verified, and the test substance was stable for the duration of the dosing period. A correction factor, based on the percent active ingredient was applied to dose formulations.
2.	Control materials	
	Negative:	0.1% Tween-80 in 0.5% aqueous methylcellulose prepared with deionized water
	Positive:	Cyclophosphamide (CP) at 40 mg/kg bw
3.	Test animals	
	Species:	Mouse
	Strain:	Crl:CD1(ICR)
	Age at dosing:	Approximately 8 weeks
	Weight at dosing:	32.5–33.2 g for males; 23.9–24.8 g for females
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	At least 5 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*.
	Water:	Tap water, *ad libitum*
	Housing:	Male animals were housed individually and female animals were housed 2-3/cage, in solid bottom cages with Shepherd's™ Cob + PLUS™ (*i.e.*, enrichment-containing bedding). Vehicle control animals were housed on a separate rack from the test substance-and CP-treated animals.
4.	Environmental conditions	
	Temperature:	18-26ºC (64-79ºF)
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	Alternating 12-hour light and dark cycles
5.	Number of animals per dose	
	Range-finding test:	3 male animals/dose
	Micronucleus assay:	5/sex/dose/sacrifice time
6	Test compound concentrations used	
	Range-finding test:	2000 mg cyantraniliprole/kg bw administered by oral gavage
	Micronucleus assay:	0, 500, 1000, and 2000 mg cyantraniliprole/kg bw administered by oral gavage

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   04-October-2010 to 02-December-2010

2. Range-finding test

   Dosing concentrations used were determined by the range finding results from an experiment using a group of 3 male animals. The highest dose used for the rangefinder for the *in vivo* micronucleus study was the limit dose of 2000 mg/kg body weight administered by oral gavage. The animals were observed for

clinical signs of toxicity and mortality immediately after dosing and daily thereafter for 2 days (until approximately 48 hours post-dosing).

3. Micronucleus assay

a Treatment and sampling times:

Test compound and vehicle control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)	[ ]	Other				
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[X]	48 hr	[ ]	72 hr

Positive control

Dosing:	[X]	Once	[ ]	twice (24 hrs apart)	[ ]	Other				
Sampling (after last dose):	[ ]	6 hr	[ ]	12 hr	[X]	24 hr	[ ]	48 hr	[ ]	72 hr

b. Tissues and cells examined**:**

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of total erythrocytes examined per animal:	1000

4. Details of slide preparation**:** Immediately after sacrifice, bone marrow was collected from both femurs per animal, aspirated and mixed with fetal bovine serum (FBS), centrifuged and placed onto slides. Bone marrow smears were stained with acridine orange, a DNA/RNA specific flurochrome. Prepared slides were coded prior to evaluation and examined for the incidence of micronucleated polychromatic erythrocytes (MPCE) and the ratio of PCE to total RBC.

5. Evaluation criteria:

Assay validity: The assay was considered valid if: 1) the mean frequency of MNPCEs in the vehicle control did not exceed 10/2000 PCEs; 2) the range of MNPCEs in the vehicle control was within the limits of the provided historical control data; and 3) the positive control induced a significant ($p \leq 0.05$) increase over the vehicle control.

Positive response: The test substance was judged positive if a statistically significant increase in the group mean MNPCEs was observed at one or more concentrations of the test substance compared to the concurrent vehicle control values with an accompanying statistically significant dose-response.

6. Statistical methods:

Data for the proportion of micronucleated PCEs among 2000 polychromatic erythrocytes and the proportion of PCEs among 1000 erythrocytes (MNPCE and PCE frequency, respectively) was transformed prior to analysis using an arcsine square root or Freeman-Tukey function. This transformation was appropriate for proportions since the distribution of the transformed data more closely approximates a normal distribution than does the nontransformed proportion. Transformed data were analysed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.

For those data that were normally distributed and had equal variance, parametric statistics (*e.g.,* analysis of variance (ANOVA) and Dunnett's test) were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal variance Dunnett's test was done. For those data that were not normally distributed, nonparametric statistics (*e.g.,* Kruskall-Wallis and Dunn's test) utilizing non-transformed data were performed. The individual animal was considered the experimental unit. All data analyses were one-tailed and conducted at a significance level of 5%.

No statistics were conducted on body weights or clinical signs.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Data from the analysis of dosing formulations for the main test were accurately prepared (98.4-100.5%), homogeneously distributed and stable at room temperature for approximately 4-5 hours.

B. RANGE-FINDING TEST

The test substance vehicle (negative) control was 0.1% Tween-80 in 0.5% aqueous methylcellulose prepared with deionized water. Three male mice were administered 2000 mg cyantraniliprole/kg by oral gavage and observed for approximately 48 hours. No clinical signs of toxicity or mortality were observed in the rangefinder experiment. On the basis of this range-finding test, the doses for the micronucleus assay were 500, 1000, and 2000 mg/kg.

B. MICRONUCLEUS ASSAY

1. Toxicity

No mortality occurred during the study. No clinical signs of toxicity were observed at any time point at any dose in male or female animals exposed to the test substance. There were no significant changes in body weight or body weight gain in either male or female animals administered the test substance.

2. PCE ratio

No depressions in the frequency of PCE/total erythrocytes (or PCE/NCE) were detected in either male or female animals exposed to the test substance.

3. Micronucleated polychromatic erythrocytes (MNPCEs)

No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance-treated group of male or female animals at the 24-hour time point or in female animals at the highest dose level at the 48-hour time point. A statistically significant increase was observed at the highest dose level in male animals at the 48-hour time point (3 MNPCEs/2000 PCEs vs. 0 in controls). However, it occurred in the absence of a statistically significant dose-response and the observed frequency was well within the historical control data of the laboratory. Therefore, it was considered spurious and without biological relevance. By contrast, the positive control (CP, 40 mg/kg) induced a significant ($p<0.05$) increase in MNPCEs/2000 PCEs. Summarized results from the micronucleus assay are presented in Tables 1 and 2.

Table 1. *In vivo* micronucleus assay in male and female mice treated with cyantraniliprole : PCE frequency

Time (h)	Sex	Number of animals	Mean PCE/NCE ratio± standard deviation				
			Vehicle control 0 mg/kg bw	Low dose 500 mg/kg bw	Mid dose 1000 mg/kg bw	High dose 2000 mg/kg bw	Positive control CP 40 mg/kg bw
24	male	5	1.10 ± 0.15	1.30 ± 0.36	1.18 ± 0.25	1.16 ± 0.19	0.94 ± 0.06
24	female	5	1.19 ± 0.13	1.19 ± 0.14	1.12 ± 0.06	1.28 ± 0.23	0.91[a] ± 0.20
48	male	5	1.35 ± 0.17	1.13 ± 0.24	1.16 ± 0.10	1.15 ± 0.24	ne
48	female	5	1.21 ± 0.15	ne	ne	1.16 ± 0.18	ne

[a] Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
CP = 40 mg/kg cyclophosphamide
Vehicle control = 0.1% Tween-80 in 0.5% aqueous methylcellulose prepared with deionized water
ne = Not evaluated

Data were derived from the study report, Tables 7 and 8, pp. 27 and 28 (MRID 48208444)

Table 2. *In vivo* micronucleus: assay in male and female mice treated with cyantraniliprole MNPCE frequency

Time (h)	Sex	Number of animals	Mean micronucleated PCE/2000 PCE ± standard deviation				
			Vehicle control 0 mg/kg bw	Low dose 500 mg/kg bw	Mid dose 1000 mg/kg bw	High dose 2000 mg/kg bw	Positive control CP 40 mg/kg bw
24	male	5	1.0 ± 1.0	1.0 ± 1.0	1.2 ± 0.8	1.8 ± 2.5	21.4[a] ± 7.1
24	female	5	2.0 ± 2.0	1.0 ± 1.4	1.4 ± 1.7	1.2 ± 0.4	20.8[a] ± 2.9
48	male	5	1.0 ± 1.0	0.2 ± 0.4	0.4 ± 0.5	3.0[a] ± 0.7	ne
48	female	5	1.2 ± 1.1	ne	ne	1.4 ± 2.1	ne

[a] Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
CP = 40 mg/kg cyclophosphamide
Vehicle control = 0.1% Tween-80 in 0.5% aqueous methylcellulose prepared with deionized water
ne = Not evaluated

Data were derived from the study report, Tables 7 and 8, pp. 27 and 28 (MRID 48208444)

III. EVALUATION, SUMMARY and CONCLUSIONS by REGULATORY AUTHORITY

A. NAME OF AUTHORITY: Health Effects Division/Office of Pesticides Program/U. S. EPA

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Totally reliable

This study is fully compliant with OECD 474 (1997)

C. CONCLUSIONS:

Cyantraniliprole was not overtly toxic or cytotoxic to the bone marrow and did not induce an increase in micronuclei in bone marrow cells of mice at doses up to the limit dose of 2000 mg/kg (oral gavage). We agree with the investigators that the slight but significant increase in MNPCE/2000 PCEs in the 48-hour sample time at 2000 mg/kg cyantraniliprole (3.0 vs. 1.0 MNPCE/2000 PCEs in the vehicle control) is well within the historical control range of 0-8 MNPCE/2000 PCEs, and is considered an anomalous finding. The sensitivity of the test system to detect a biologically relevant and significant ($p<0.05$) increase in MNPCEs was adequately demonstrated by the results obtained with the positive control, CP. It should be noted that an error was found in the table heading for Table 2. Mean Micronucleated PCEs were presented per 1000 PCEs. This error, which was corrected by the reviewers, now reads: Mean Micronucleated PCE/2000 PCEs.

Accordingly, Cyantraniliprole is considered negative in this *in vivo* test system**.**

EPA Reviewer: Whang Phang, PhD **Signature:**
RAB 3, Health Effects Division (7509P) **Date:** 11/10/2012
EPA Secondary Reviewer: Anwar Dunbar, PhD **Signature:**
RAB 1, Health Effects Division (7509P) **Date:** 11/10/2012

TXR: 0056591

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (28-day) Inhalation Toxicity - rats; OPPTS 870.3465 [§82-4]; OECD 413.

PC CODE: 090098 **DP BARCODE: D**

TEST MATERIAL (PURITY): Cyantraniliprole (DPX-HGW86); 3-Bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide (95.6% purity)

SYNONYMS: 3-Bromo-N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloropyridinyl)-1Hpyrazole-5-carboxamide (Alternative IUPAC name)

CITATION: Ng, Sheyng P. (2011) Cyantraniliprole (DPX-HGW86) Technical: Four-Week Inhalation Toxicity Study in Rats. DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, Delaware 19714, U.S.A. Laboratory report No. DuPont-32967. November 18, 2011. MRID 48663602. Unpublished

Bentley, KS and Delorme, MP (2012) Position Paper: Cyantraniliprole: Additional information in support of DuPont-32967, Four-Week Inhalation toxicity study in rats. DuPont Crop Protection, Stine Haskell Research Center, 1090 Elkton Rd., Newark, Delaware 19711, USA. DuPont Project identification : DuPont-35858. August 10, 2012. MRID 48894806. (Supplemental Submission) Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 48663602), Cyantraniliprole (DPX-HGW86) Technical (95.6% purity; lot/batch #HGW86D008A; Haskell number 29548) was administered (as aerosol) by inhalation (nose only) to male and female Crl:CD(SD) albino rats (10/sex/dose) for 6 hours/day, 5 days/week, for 4-weeks. The test animals were exposed nose only to target concentrations of 0 (air control), 1, 10, or 100 mg/m³ of cyantraniliprole. Additional two groups of 10/sex/dose served as recovery groups which received 0, or 100 mg/m^3 for 4 weeks and followed by a 2 weeks recovery period. The targeted concentrations, 1, 10, and 100 mg/m^3 are equivalent to 0.001, 0.01, and 0.1 mg/L, respectively.

The results showed that rats from the 1 mg/m³ group were exposed to a gravimetrically determined aerosol concentration of 1.4 mg/m³ with a mass median aerodynamic diameter (MMAD) of 2.9 μm and geometric standard deviation (GSD) of 2.3. Rats from the 10 mg/m³ target level were exposed to 11 mg/m³ with a MMAD of 2.5 μm and GSD of 2.4. Rats from the 100 mg/m³ target level were exposed to 100 mg/m³ with a MMAD 3.0 μm and a GSD of 2.3.

Cyantraniliprole did not produce compound-related effects on body weights, body weight gains, food consumption, food efficiency, or ophthalmological observations. No clinical signs of toxicity were observed over the course of this study. There were no adverse changes in clinical pathology parameters. No test substance-related changes in organ weights or gross observations were found.

Histopathology examination showed an increase in the incidence of focal squamous metaplasia of the larynx (8/10) in the 100 mg/m³ males No increase was seen in females. In the recovery phase no increase was seen in males, but 1 female in the 100 mg/m^3 group had laryngeal focal squamous metaplasia. The laryngeal focal squamous metaplasia seen in all affected animals was graded as minimal effect. The published literatures [1 & 2] showed that laryngeal squamous metaplasia was frequently observed in rodent inhalational studies after exposure to a variety of test compounds and even by "non-chemical" means such as irritation by aerosols and particles, and dehydration by alcohols or low humidity air." In addition, "there is no published evidence that this effect is pre-neoplastic and it is repeatedly characterized as an adaptive response" [2]. The data of the recovery group showed no laryngeal squamous metaplasia and lack of progression over time support reversibility of this effect. Therefore, the increase in the incidence of minimal laryngeal squamous metaplasia in this study is considered to be treatment-related, but it is not adverse. However, with longer duration of exposure or higher concentrations, the effects seen in the larynx could potentially progress and become adverse.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for cyantraniliprole was 100 mg/m³ (0.1 mg/L) (highest exposure concentration tested) in male and female rats. No LOAEL could be established.

The results of this subchronic inhalation toxicity study in Crl:CD(SD) albino rats are considered reliable (Acceptable/non-guideline) and satisfies most of the requirements for a 28-day inhalation toxicity study (US EPA guideline 870.3465 [§82-4]; OECD 413).

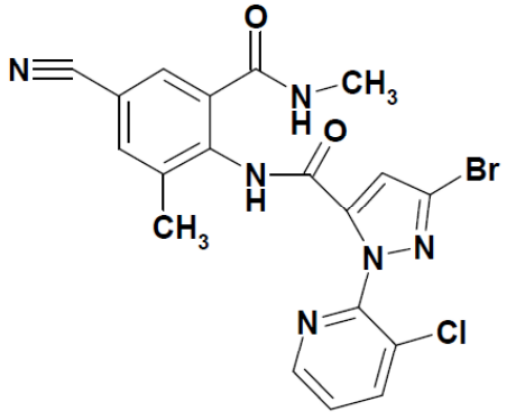
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cyantraniliprole (DPX-HGW86-586) Technical

Description: 3-Bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide; off white solid/powder



Lot/batch #:	HGW86D008A; Haskell number 29548
Purity:	95.6 % .
Compound stability:	Stable at ambient temperature
CAS # of TGAI:	736994-63-1

2. Vehicle and/or positive control: No applicable

3. Test animals:

Species:	Albino rats
Strain:	Crl:CD(SD)
Age/weight at study initiation:	≈ 8 weeks old; between 251-314 gm males; 188-237 gm females
Source:	Charles River Laboratories,Inc., Kingston, New York
Housing:	Animals were housed in pairs (sexes separated) in solid bottom caging with Sheperd'sTM Cob + PLUSTM as bedding and enrichment
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum* (except during exposure)
Water:	Tap water *ad libitum*
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** 12 air exchanges/hour **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	≈6 days

B. STUDY DESIGN:

1. In life dates: Start: May 17, 2011; end: June 30, 2011

2. **Animal assignment:** Animals were assigned by computerized, stratified randomization into study groups as designated in the Study Design, (i.e., n=20, 10, 10, 20 rats/sex for target exposures to 0, 1, 10, or 100 mg/m3 cyntraniliprole, respectively) so that there were no statistically significant differences among group body weight means within a sex. The weight variation of selected rats did not exceed ± 20% of the mean weight for each sex. The first 10 rats in each group were designated for toxicity evaluation, and the remaining rats in the control and high concentration group were designated for recovery as summarized below:

		Number of Animals			
	Targeted Atmospheric	Cohort I: Toxicity		Cohort II: Recovery	
Group	Concentration (mg/m^3)	Males	Females	Males	Females
1	0 (Control)	10	10	10	10
2	1	10	10		
3	10	10	10		
4	100	10	10	10	10

The schedule for study functions is as follows:

	Approximate Study Week						
Function	Pretest	1	2	3	4	5[a]	6
Body Weight	≥ 3 times ←	twice / week →				←once/week→	
Daily Animal Health Observations	← Once Daily →						
Post-Exposure Clinical Observation	← Following Exposures →						
Detailed Clinical Observation	← Weekly →						
Food Consumption	← Weekly →						
Ophthalmology Evaluation	[b]	-	-	-	[c]	-	-
Clinical Pathology Evaluation	-	-	-	-	[d, e]	-	[e]
Anatomic Pathology Evaluation	-	-	-	-	[d]	-	[e]

a Beginning of recovery period.
b Conducted on all animals received for the study.
c Conducted on all surviving animals.
d Conducted on 10 animals per group designated for toxicity evaluation.
e Conducted on 10 animals per group designated for recovery evaluation. For clinical pathology evaluation, samples were only analyzed for selected parameters.

.

TABLE 1: Study design: nominal and analytical concentrations[a], MMAD, and GSD

Test group	Nominal conc. (mg/m^3)	Analytical Conc. [a] (mg/ m^3)	MMAD µm	GSD	Rats/sex
Control	0				10
Low (LCT)	1.0	1.41±0.13	2.9	2.3	10
Mid (MCT)	10	11.0±0.53	2.5	2.4	10
High (HCT)	100	100±1.0	3.0	2.3	10

Test group	Nominal conc. (mg/m^3)	Analytical Conc. [a] ($mg/ m^{3)}$	MMAD µm	GSD	Rats/sex
Recovery group	0 and 100	100±1.0	3.0	2.3	10

[a]: Analytical concentrations were determined by gravimetric analysis.

Recovery groups: At the conclusion of the 4-week exposure period (i.e., test days 0-29), all rats designated as recovery groups (10/sex from control and high-concentration group) were allowed to recover for approximately 2 weeks. During the recovery period (i.e., test days 30-44 for males and days 30-43 for females), exposures were not conducted; however, in-life observations and measurements (clinical observations, body weights, and individual food consumption) were continued. Prior to the end of the recovery period, all surviving rats underwent a clinical pathology evaluation on selected parameters. At the conclusion of the recovery period, surviving rats were sacrificed, and tissues collected for possible microscopic evaluation.

3. **Dose selection rationale:** The report stated that a pilot study in which 3 males and 3 females were exposed to the highest test concentration (100 mg/m^3) for 3 days (6 hr/day) was performed (in a non-GLP manner) to ensure the high concentration level chosen for this study did not result in overt or excessive toxicity. The data of this pilot study were not included in the study report and it was not clear why the highest concentration was set at 100 mg/m^3. The registrant later provided a position paper concerning the dose selection, but it contained no experimental data (please see Appendix 1)

4. **Generation of the test atmosphere / chamber description:**. Test atmospheres were generated by suspension of cyantraniliprole in air with a Fluid Energy Processing model 00 jetmill. The test substance was metered into the jetmill with a Schenck/Accurate model 102 Single Screw volumetric feeder for the 1 and 10 mg/m^3 chambersand a K-Tron model T-20 Twin Screw volumetric feeder for the 100 mg/m3 chambers. High pressure air, metered into the jetmill by a jet-o-mizer, carried the resulting atmosphere into the exposure chamber. Chamber concentrations of test substance were controlled by varying the test substance feed rate to the jet mills. Atmospheric concentrations of test substance were determined by gravimetric analysis and the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol atmosphere was determined with an 8-stage Sierra® cascade impactor (Table 1). The schematic of the exposure system is presented in Figure 1 of the Appendix 2.

   The overall chamber temperatures for all exposure groups ranged from 20-25°C and the relative humidity (RH) ranged from 40-65%. There was 30 L/min airflow through the chambers which provided 12 air changes per hour. The oxygen concentration in the chambers was 20-21%. The chamber environmental conditions were considered adequate for the conduct of the study.

5. **Duration of Exposure:** Each group of animals was exposed nose only for 6 hours/day, 5 days/week, over a 4-week period (weekends and holidays excluded) for a total of 21 exposures (during test day 0-29). To accommodate the laboratory facilities schedule, the initiation of exposures was staggered by one day. Due to the staggered start, animals received a partial week of exposures during the first and last weeks of the study. However, the total number of exposures was 21.

6. Statistics: The statistical methods and analyses are summarized below:

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Exposure Concentration Environmental Monitoring	None	Descriptive statistics (e.g., mean, standard deviation)	
Body Weight Body Weight Gain Food Consumption Food Efficiency Clinical Pathology[a] Organ Weight	Levene's test for homogeneity[4] and Shapiro-Wilk test[5] for normality[b]	One-way analysis of variance[6] followed by Dunnett's test[7,8,9]	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

a When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1, 0.05 was used for any calculations performed with that bilirubin data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with that data.

b If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected daily for signs of toxicity and mortality.

1b. Clinical examinations: Detailed clinical observations, including, (but not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, , changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behavior, were conducted approximately once a week during both exposure and recovery periods. Any abnormal clinical signs noted were recorded.

1c. Neurological evaluations: The following evaluations (measurements) were performed on day once a week: autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern).

2. Body weight: Test animals were weighed before the exposure period, at least twice a week during the exposure period, and at least once a week during the recovery period. At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. At necropsy, final body weights were taken for all rats sacrificed by design.

3. **Food consumption and food efficiency:** The amount of food consumed by each animal over the weighing interval was determined weekly by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight divided by the number of animals in the cage. From these measurements, mean daily food consumption over the interval was determined. From the food consumption and body weight data, the mean daily food efficiency was calculated.

4. **Ophthalmoscopic examination:** Two ophthalmology examinations were conducted by a veterinary ophthalmologist. The pretest examination was performed on all rats received for the study, prior to assignment to groups. Any rats with preexisting ophthalmology abnormalities were eliminated from consideration for use in the study. All surviving rats were examined prior to the end of the exposure period (i.e., on test day 24 for males and day 23 for females). Both eyes of each rat were examined by focal illumination and indirect ophthalmoscopy. The eyes were examined in subdued light after mydriasis had been produced.

5. **Hematology and clinical chemistry:** Blood samples for hematology and clinical chemistry measurements were collected from the tail vein of each animal while the animal was under isoflurane anesthesia. A second clinical pathology sample collection was conducted at the end of the recovery period (i.e., on test day 44 for males and day 43 for females) in recovery animals. Blood samples for coagulation parameters were collected at sacrifice (i.e., day 30 for toxicity animals and day 44 or 43 for recovery animals) from the abdominal *vena cava* of each animal while the animal was under isofurane anesthesia. Additional blood collected from the *vena cava* was placed in a serum tube, processed to serum, and frozen at approximately -80°C.Blood was collected. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	x	Leukocyte differential count*
X	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
X	Platelet count*	x	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
X	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	x	Albumin*
X	Chloride	x	Creatinine*
	Magnesium	x	Urea nitrogen*
X	Phosphorus	x	Total Cholesterol*
X	Potassium*	x	Globulins
X	Sodium*	x	Glucose*

X	ELECTROLYTES	X	OTHER
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	x	Total bilirubin
X	Alkaline phosphatase*		Total serum protein (TP)*
	Cholinesterase (ChE)	x	Triglycerides
	Creatine Phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urine was collected from fasted animals at 1 day after the exposure period. The CHECKED (X) parameters were examined.

X	Appearance* (clarity, color, quality)	x	Glucose*
X	Volume*	x	Ketones
X	Specific gravity / osmolality*	x	Bilirubin
X	pH*	x	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	x	Urobilinogen

* Optional for inhalation toxicity studies

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct* (rat)
X	Pancreas*

X	RESPIRATORY
X	Trachea*
Xx	Lung*
X	Nose* (4 sections)
X	Pharynx*
Xx	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
Xx	Heart*+
X	Bone marrow*
X	Lymph nodes*
Xx	Spleen*+
Xx	Thymus*+

X	UROGENITAL
Xx	Kidneys*+
X	Urinary bladder*
Xx	Testes*+
Xx	Epididymides*+
X	Prostate*
X	Seminal vesicles*
Xx	Ovaries*+
Xx	Uterus*+
Xx	Mammary gland*

X	NEUROLOGIC
xx	Brain*+
x	Peripheral nerve* (sciatic)
x	Spinal cord (3 levels)*
x	Pituitary*
x	Eyes (optic nerve)*

X	GLANDULAR
xx	Adrenal gland*+
	Lacrimal gland
x	Parathyroid*
x	Thyroid*

X	OTHER
x	Bone (sternum and/or femur)
	Skeletal muscle
x	Skin
x	All gross lesions and masses*

* Recommended for subchronic rodent studies based on Guideline 870.3465

+ Organ weights required

II. RESULTS:

A. OBSERVATIONS :

1. Clinical signs of toxicity: There were no test substance-related clinical observations of toxicity in any of the exposure groups during the 6-hour exposures, immediately following the exposures, at cage-site evaluations or detailed clinical observations during the exposure or recovery periods. However, 1 female of the 100 mg/m^3 group showed signs of gasping and lung noise during breathing on day 23. Salivation was also seen in this animal at that day. This incidence could not be considered as compound-related because it was seen in a single day and low incidence; no other correlated changes were reported in this animal.

2. Mortality: All animals survived to scheduled necropsy.

3. Neurological evaluations: No neurological signs were reported for any test group.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weights and body weight gains during 4 weeks of treatment are presented in Table 2. Sporadic reduction in body weights gains were seen in 100 mg/m^3 male and 1.4 mg/m^3 females. Some of these changes demonstrated a statistically significant difference from the controls. However, the periodic reductions in body weight gains were not considered as toxicologically significant as the magnitudes of changes were small. The two-week recovery groups showed no significant difference between the control and highest concentration groups (Table 3).

Table 2. Body weights and body weight gains for animals in the main study.

Concentration (mg/m^3)	Body weights (gm)				Total weight gain	
	Week -1 (Day 0)	Week 1 (Day 7)	Week 2 (Day 14)	Week 4 (Day 28)	gm (Day 0-30)	% from control (Day 0-30)
Male						
0	280±18	319±19	365±25	426±31	114	
LCT (1)	285±8.3	329±15	351±47	424±30	105	-8
MCT (10)	284±12	329±13	371±15	430±16	107	-6
HCT(100)	282±18	322±21	360±24	415±26	98	-14
Female						
0	209±13	225±13	243±15	260±18	27	
LCT(1)	206±8.1	225±8.7	240±10	262±15	33	25
MCT(10)	212±8.8	228±9.4	238±13	258±23	17	-35
HCT(100)	209±12	224±13	238±16	256±20	24	-8

[a] Data obtained from Tables 4, 5, 6, & 7, pages 39 – 48 in the study report.
* Statistically different ($p < 0.05$) from the control. ** Statistically different ($p < 0.01$) from the control.

Table 3. Body weights and body weight gains for animals in the recovery groups.

Concentration (mg/m^3)	Body weights (gm)		Total weight gain	
	Week 5 (days 35/36; F/M)	Week 6 (Days 42/43; F/M)	gm (day 0-44 for M) (Day 0-43 for F)	% from control (Day 0-44 for M) (Day 0-43 for F)
Males				

0	443±36	481±40	165	
HCT (100)	428±25	471±30	159	-3.4
Females				
0	263±16	277±19	46	
HCT (100)	267±19	284±21	47	1

M= males; F=females Data excerpted Tables 4, 5, 6, & 7, pages 39 – 48 in the study report.

C. FOOD CONSUMPTION:

1. **Food consumption:** The data demonstrate no compound related effects on food consumption.

2. **Food efficiency**: Food efficiency was not affected by the compound.

D. OPHTHALMOSCOPIC EXAMINATION: Only one (of twenty) female in 100 mg/m^3 group was identified with unilateral retinal degeneration of unknown etiology prior to the end of exposure period. This effect was not found in any test animals of the other groups. Therefore, this finding was not treatment-related.

E. BLOOD ANALYSES:

1. **Hematology:** There were no dose-related changes in group mean hematology parameters in male or female rats.

2. **Clinical chemistry:** There were no treatment-related and adverse changes in group mean clinical chemistry parameters in male or female rats. There was a minimal and statistically significant reduction in serum bilirubin in 100 mg/m^3 males and females. However, following the 2-week recovery period, group mean serum bilirubin concentrations were similar to the respective control groups and were not statistically significant. Based on the minimal nature and direction (decreased rather than increased), the lower serum bilirubin in the 100 mg/m^3 group was considered not adverse.

Table 4. Selective clinical chemistry data (bilirubin, mg/dL)

Duration of the study (days)	Concentration (mg/m^3)			
	0	1	10	100
Males				
30 (main study)	0.11±0.02	0.11±0.02	0.12±0.01	0.10±0.01* (↓10%)
44 (recovery group)	0.13±0.01			0.12±0.02
Females				
30 (main study)	0.13±0.02	0.14±0.02	0.13±0.02	0.10±0.02* (↓23%)
44 (recovery group)	0.14±0.02			0.13±0.01

*: Statistically significant ($p<0.05$) Data excerpted from pages 67 & 70 of the study report.

F. URINALYSIS: There were no compound-related changes in group mean urinalyses parameters in male or female rats.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were no compound-related organ weight changes. However, lower spleen weight was observed in the 100 mg/m^3 male at the end of the exposure period (Table 4). These spleen weight changes showed statistically significant difference from the controls, but they were not associated with correlative pathological changes in the spleen or other lymphoid organs, and were not observed in the 100 mg/m^3 male recovery or any female group. Therefore, the spleen weight reduction was not considered as a compound-related finding.

Table 4. Absolute and relative spleen weights in male rats

Spleen weights	Concentration (mg/m^3)			
	0	1	10	100
Absolute (gm)	0.712±0.086	0.693±0.084	0.654±0.067	0.610±0.077* (↓14%)
Spleen/body weight (%)	0.18	0.18	0.17	0.16*
Spleen/brain weight (%)	35	34	32	30*

*: statistically significant ($p<0.05$) Data excerpted from page 78 of the study report.

2. **Gross pathology:** There were no test substance-related gross observations in this study.

3. **Microscopic pathology:** Minimal, focal squamous metaplasia was observed in the ventral laryngeal mucosa of 8/10 males in the 100 mg/m^3 group (Table 5). This change was limited to the laryngeal section taken at the base of the epiglottis, as no changes were observed in the second laryngeal (more caudal) section taken at the level of the laryngeal pouch. Squamous metaplasia was not associated with changes in the underlying submucosa, submucosal glands, or associated laryngeal cartilage. In males, the laryngeal change was reversible, as no exposure-related microscopic changes were observed in the larynx of rats in the 100 mg/m^3 male recovery group; but it was seen in a single female of 100 mg/m^3 level.

Table 5. Incidence of minimal focal squamous metaplasia in the laryngeal mucosa

	Concentration (mg/m^3)			
	0	1	10	100
Males	0/10	0/10	0/10	8/10
Females	0/10	0/10	0/10	0/10
Recovery Groups				
Males	0/10			0/10
Females	0/10			1/10

Data excerpted form pages 100 & 103 of the study report.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Exposure to cyantraniliprole did not result in test substance-related adverse changes in body weights, body weight gains, daily food consumption, daily food efficiency, or ophthalmological observations, and no clinical signs

of toxicity were observed over the course of this study. There were no adverse changes in clinical pathology parameters. There were no test substance-related organ weight changes or gross observations in this study. No adverse anatomic pathological changes were observed in male or female rats exposed to cyantraniliprole at concentrations up to 100 mg/m^3. Exposure-related focal squamous metaplasia of the larynx was observed in 8/10 males in the 100 mg/m^3 group. This change was minimal and limited to the ventral laryngeal mucosa lining the base of the epiglottis. The laryngeal change was reversible [1 & 2], as no exposure-related microscopic changes were observed in the larynx of rats in the 100 mg/m^3 male recovery group. Minimal squamous metaplasia was observed in 1/10 female rat in the 100 mg/m^3 recovery group but not in the same group at the end of the exposure period. Therefore, this single instance of laryngeal squamous metaplasia was considered a spurious finding. Such minimal and focal changes in the rodent larynx are considered adaptive and non adverse. There were no other test substance-related pathology findings in male or female rats.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for cyantraniliprole was 100 mg/m^3, the highest exposure concentration tested in this study, for male and female rats.

B. <u>REVIEWER COMMENTS</u>: Cyantraniliprole (DPX-HGW86) Technical (95.6%) was administered (as aerosol) by inhalation (nose only) to male and female Crl:CD(SD) albino rats (10/sex/dose) for 6 hours/day, 5 days/week , for 4-weeks. The test animals were exposed nose only to target concentrations of 0 (air control), 1, 10, or 100 mg/m^3 of cyantraniliprole Additional two groups of 10/sex/dose served as recovery groups which received 0, or 100 mg/m^3 for 4 weeks and followed by a 2 weeks recovery period.

The results showed that rats from the 1 mg/m^3 group were exposed to a gravimetrically determined aerosol concentration of 1.4 mg/m^3 with a mass median aerodynamic diameter (MMAD) of 2.9 μm and geometric standard deviation (GSD) of 2.3. Rats from the 10 mg/m^3 target level were exposed to 11 mg/m^3 with a MMAD of 2.5 μm and GSD of 2.4. Rats from the 100 mg/m^3 target level were exposed to 100 mg/m^3 with a MMAD 3.0 μm and a GSD of 2.3.

Cyantraniliprole did not produce compound-related effects on body weights, body weight gains, food consumption, food efficiency, or ophthalmological observations. No clinical signs of toxicity were observed over the course of this study. There were no adverse changes in clinical pathology parameters. No test substance-related changes in organ weights or gross observations.

Histopathology examination showed an increase in the incidence of focal squamous metaplasia of the larynx (8/10) in the 100 mg/m^3 males. No increase was seen in females. In the recovery phase no increase was seen in males, but 1 female in the 100 mg/m^3 group had laryngeal focal squamous metaplasia.

By definition, squamous metaplasia refers to benign (non-cancerous) changes in the epithelial linings of certain organs within the body. Smokers often exhibit squamous metaplasia in the

linings of their airways. These changes don't signify a specific disease, but rather usually represent the body's response to stress or irritation.

The published literatures [1, 2] showed that laryngeal squamous metaplasia was frequently observed in rodent inhalation studies after exposure to a variety of test compounds and even by "non-chemical" means such as irritation by aerosols and particles, and dehydration by alcohols or low humidity air." In addition, "there is no published evidence that this effect is pre-neoplastic and it is repeatedly characterized as an adaptive response"[2]. The data of the recovery group showed no laryngeal squamous metaplasia and lack of progression over time support reversibility of this effect. Therefore, the increase in the incidence of minimal laryngeal squamous metaplasia in this study is considered to be treatment-related, but it is not adverse under the current conditions.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for cyantraniliprole was 100 mg/m^3 (0.1 mg/L), the highest exposure concentration tested (HCT) for male and female rats.

The results of this study are considered reliable (Acceptable/non-guideline) and satisfies most of the essential requirements for a 28-day inhalation toxicity study (US EPA guideline 870.3465 [§82-4]; OECD 413).

References

1 Kaufmanna W, Bader R, et al. (2009). 1st International ESTP Expert Workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. Experimental and Toxicologic Pathology. 61 (6):591-603.

2 Osimitz TG, Droege W, and Finch, JM (2007). Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: A critical review. Toxicology and Applied Pharmacology, 225:229–237.

Appendix 1

Evaluation of the Supplemental Information in Support of the 28-Day Inhalation Toxicity Study Submitted by DuPont Crop Protection; DuPont Project Identification: DuPont-35858. MRID 48894806

A pilot study was conducted on a group of 3 males and 3 females Crl:CD(SD) rats. The test animals were exposed to a target concentration of 100 mg/m^3 of cyantraniliprole by nose only for 6 hours/day for 3 days. The data indicated that test rats were exposed to cyantraniliprole at 100±7.6 mg/m^3 which was characterized by a particle size distribution with MMAD of 3.0 (GSD 2.2). No adverse effects were found during the exposure period or subsequent to exposure period (12 days following the final exposure). Based on the results of the pilot study, 1, 10, and 100 mg/m^3 were selected for the 28-day inhalation study. In the main study, no adverse effect was seen at the highest concentration (100 mg/m^3) tested. However, reversible squamous cell metaplasia was found in the larynx of male rats; the effect was graded as minimal and was absent in the recovery animals following the 2 weeks of recovery period.

The registrant provided addition information on the pilot study and the rationale for selecting 100 mg/m^3 as the highest concentration for the 28-day inhalation study. The two reasons provided by the registrant were not based on generated data. The first reason was that cyantraniliprole was barely soluble in water (~0.001%) and as such that cyantraniliprole was not expected to dissolve in fluids lining the lung or to be absorbed by the lung in any significant degree. In addition, 100 mg/m^3 was considered by the registrant to be a high concentration for occupational exposure and the registrant further argued that highest occupational exposure to any poorly soluble particulate in the workplace typically recommended was 10-15 mg/m^3 total dust and 3-5 mg/m^3 respirable fraction (20 CFR 1910.1001; ACGIH 2001). The registrant felt that the highest concentration tested was at least 7 times the recommended particulate concentration. US EPA believes that range of 10-15 mg/m^3 concentration pertains to a close system such as factory, whereas the occupational exposure resulting from cyantraniliprole proposed use is in the open air. Therefore, this reason is not applicable.

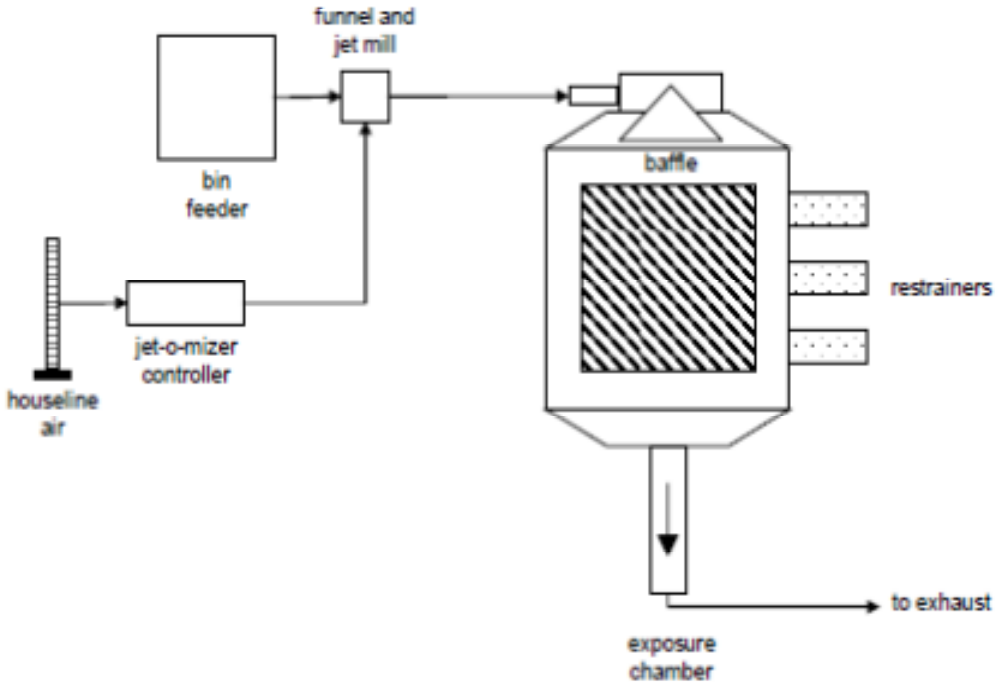
The second reason provided by the registrant was that with atmospheres above 100 mg/m^3, it could be difficult to maintain particle size distributions such that the MMAD was below 3 µm. The registrant added that aerosols above 100 mg/m^3, in general, resulted in an increase in inter-particle collisions and resulting in agglomeration which would lead to increased MMADs. The increases in MMADS would potentially reduce the deposition of particles into the deeper regions of the lung. This argument sounds plausible but there is no data to substantiate the proposed reasoning.

After evaluating the additional information on the pilot study and the results of the main 28-day inhalation toxicity study, US EPA believes the finding of the reversible metaplasia of squamous cell of the larynx at 100 mg/m^3 indicate that with either higher cyantraniliprole concentrations or longer duration of exposure, the effects seen in larynx would potentially progress and become adverse. Therefore, the NOAEL for the 28-day inhalation toxicity is established as 100 mg/m^3 (0.1 mg/L), and the study is reliable (acceptable/non guideline).

Appendix 2

(Figure excerpted from page 105 of the study report)

Figure 1
Schematic of Exposure System



Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6.1 Dermal absorption, *in vivo* in the rat

Report: Fasano, W.J. (2008); Cyantraniliprole (DPX-HGW86) 100 g/L OD: *In vivo* dermal absorption of cyantraniliprole in rat. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-24503. December 17, 2008. MRID 48120209. Unpublished.

Guidelines: OECD, Part 427 (2004)
OECD 28 (2004),
ECC Sanco/222/2000 Rev.7 (2004)
Deviations: None

GLP: Yes. Signed statements of GLP Compliance, Data Confidentiality, and Quality Assurance were submitted in the study report.

Executive summary:

An *in vivo* dermal absorption study on cyantraniliprole oil dispersion (OD) formulation was conducted in Crl:CD®(SD)IGS BR rats (8 males/group) (MRID 48120209). The test substance, Cyantraniliprole 100 g/L OD, was applied as the undiluted concentrate at 100 g cyantraniliprole/L and as an aqueous dilution at 1 g cyantraniliprole/L. Absorption was followed using [^{14}C]-cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied to a 10.5 cm² shaved area on the dorso-lumbar region at a rate of 10 µL/cm² to 2 groups of 4 rats per dose level. The amount of cyantraniliprole applied per area of skin was approximately 1000 µg/cm² and 10 µg/cm² for the 100 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skin for 6 hours. At 6 hours, the skin surface of all rats was washed with a dilute soap solution and one group of 4 rats per dose concentration was sacrificed to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining 4 rats at each dose level were maintained until 504 hours post-dose (498 hours post-exposure; 21 days). At sacrifice, the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The report stated that formulation concentrations and application rates were designed to mimic potential field-use exposures.

For the undiluted concentrate, during the 6-hour dermal exposure only a negligible amount of the applied dose was absorbed (0.36%). At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (88.6%), while removal of the stratum corneum by tape stripping accounted for approximately 3.43%. Given the amount of dose absorbed (0.36%) and the dose remaining in the tape-stripped skin (0.36%), the total absorbable dose at 6 hours was calculated to be 0.72%. **Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.36% of the administered dose.**

For the 1 g/L aqueous dilution of the Cyantraniliprole 100 g/L OD formulation, over the course of a 6-hour dermal exposure, only 0.18% of the applied dose was absorbed. At the end of

the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>86%), while removal the stratum corneum by tape stripping accounted for 4.44%. Given the amount absorbed (0.18%) and the dose remaining in the tape-stripped skin (0.30%), the total absorbable dose at 6 hours was calculated to be 0.47%. **Based on a 498-hour recovery/ collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.74% of the administered dose.**

In summary, following a 6-hour *in vivo* dermal exposure to Cyantraniliprole 100 g/L OD formulation, when applied either as the undiluted concentrate or as an aqueous dilution, the maximal absorbable dose was 1.36% and 0.74% of the administered dose for undiluted formulation and aqueous dilution, respectively. The dermal absorption data from both *in vivo* and *in vitro* dermal absorption studies were used to estimate the *in vivo* dermal absorption factor for human; the estimated factors were 0.06% for cyantraniliprole 100 g/L OD formulation concentrate and 0.03% for aqueous dilution.

This study is reliable (acceptable) and meets the data requirements for an in vivo dermal absorption study (OECD, Part 427 (2004)) on formulation.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 100 g/L OD
	Lot/Batch #:	HGW86-329
	Purity:	100 g ai/L (undiluted concentrate) 1 g ai/L (field application rate)
	Description:	Liquid
	CAS #:	None for the test formulation 736994-63-1 for the active ingredient
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantraniliprole
	Lot/Batch #:	[pyrazole carbonyl-^{14}C[-cyantraniliprole abbreviated as [PC-^{14}C]-cyantraniliprole, Lot #: 3562-042
	Radiochemical purity:	[PC-^{14}C]-cyantraniliprole: radiochemical purity >98%
	Specific activity:	[PC-^{14}C]-cyantraniliprole: 44.06 μCi/mg
	Description:	Not specified in report
	Stability of test compound:	The test materials were stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of cyantraniliprole, was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated

		formulation. The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water for the aqueous dilution
5.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)IGS BR
	Sex	Males
	Age at dosing:	Approximately 6-8 weeks old
	Weight at dosing:	199-220 g
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	6 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in all-glass metabolism units
4.	Environmental conditions	
	Temperature:	22-24°C
	Humidity:	40-60%
	Air changes:	Not reported
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

19-August-2008 to 1-October-2008

2. Animal assignment and treatment

On the day prior to dermal dosing, the back and shoulders of each animal was clipped free of hair and the clipped area washed with a 2% Ivory® Soap solution in water. Following shaving and washing, three O-rings (one stacked upon the other) with an internal area of approximately 10.5 cm2, were glued to the clipped area on the back using Instant Krazy Glue® Gel. The O-rings were then covered with Coban™ body wrap to prevent contamination

Doses applied and target parameters are summarised in Table .

Table 1
Study design: Summary of doses applied and target parameters for the *in vivo* assessment of dermal absorption of cyantraniliprole in the rat

Group	Dose concentration of cyantraniliprole	Skin dose level of cyantraniliprole	Number of animals[a]	µCi/rat
A	100 g/L[b]	1000 µg/cm^2	8	8
B	1 g/L[c]	10 µg/cm^2	8	5

[a] Based on 4 rats at 2 post-exposure collection timepoints

[b] Rats in group A were exposed to a single application of the undiluted formulation concentrate.

[c] Rats in group B were exposed to a single application of an aqueous dilution of the formulation.

Two sacrifice time intervals for Group A and Group B treatment groups were included in this study:

Interval I: 4 animals were sacrificed 6 hours after dermal application (0 hours post-exposure).

Interval II: 4 animals were sacrificed 504 hours after dermal application (498 hours post-exposure).

3. Dose formulation and analysis

The Cyantraniliprole 100 g/L OD undiluted concentrate was prepared by mixing radiolabeled cyantraniliprole (~5.8 mg) with non-radiolabeled cyantraniliprole (~330 mg) followed by the addition wet-milled at approximately 3000 rpm for 50 minutes. The wet-milled ingredients were separated from the glass beads by syringe and placed into a glass vial.

The Cyantraniliprole 100 g/L OD aqueous dilution was prepared by mixing radiolabeled cyantraniliprole (~3.4 mg), formulation blank (~30 mg), and deionized water (~3.3 g) with glass beads (5.4 g @ 0.5-0.75 mm). The ingredients were wet-milled at approximately 3000 rpm for 50 minutes. The aqueous dilution was separated from the glass beads by syringe and placed into a glass vial.

The formulated products were stored frozen at <-10°C.

The homogeneity and amount of radiolabeled cyantraniliprole (µCi/g) in each formulated dose was determined by subjecting aliquots of the prepared dose to radioanalysis by liquid scintillation counting (LSC). The concentration of cyantraniliprole in each dose formulation was determined chromatographically by HPLC-UV. The results of homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled cyantraniliprole (µCi/mg) for the formulated doses. The radiochemical purity of the neat radiolabeled cyantraniliprole and the stability of radiolabeled cyantraniliprole in the prepared dose formulations were determined by HPLC-radiochromatography.

4. Dosing

On the day of dosing, the protective gauze wrap was removed and the formulated products were applied within the O-ring area at a rate of 10 μL/cm^2. Following dosing, the dose site was protected with a rigid mesh covering and Coban™ body wrap and each animal was separately housed in an all-glass metabolism cage. The applied formulation remained in contact with the skin for 6 hours. After 6 hours, the skin surface of all rats was washed using natural sponge pieces soaked in 2% Ivory® soap and water.

5. In-life sample collection

Urine and faeces were collected during the 0-6 hour exposure period, at 6-12 hours, 12-24 hours and every 24 hours thereafter until sacrifice. The 6-hour aqueous soap solution washes of the application site, the sponges used during the washing, and the body wrap and rigid mesh covering (from the end of the exposure phase and at sacrifice) were collected for analysis.

6. Sacrifice

Animals were exposed to carbon dioxide asphyxiation and exsanguinated *via* cardiac puncture. The application skin site was excised and tape-stripped to remove the stratum corneum. The skin washes, Coban™ body wrap, cage washes, residual feed, skins from the application site, tape strips, skins from a non-dosed area, blood (whole blood, plasma, and red blood cells), and remaining carcass were analysed for total radioactivity.

7. Sample analysis

Urine, skin washes, cage washes, blood plasma were assayed directly by LSC. The protective mesh cover, Coban™ body wrap, O-ring spacers, and tape strips were extracted using acetonitrile and analysed directly by LSC. Faeces, residual feed, carcasses were homogenised in water and combusted prior to LSC. Skin samples (both the application skin and non-dose skin) and wash sponges pieces were digested in Soluene®-350 and analysed directly by LSC.

8. Statistics: Group data is presented as the mean result.

II. RESULTS AND DISCUSSION

A. RADIOCHEMICAL PURITY, CONCENTRATION AND STORAGE STABILITY

The purity of radiolabeled cyantraniliprole was >95%. Analyses confirmed that cyantraniliprole was present in the dosing formulations at the appropriate concentrations and with the appropriately amount of radiolabeled test substance. [^{14}C]-cyantraniliprole and the formulations were shown to be stable under the conditions of this study.

B. TREATMENT GROUP A – CYANTRANILIPROLE OD UNDILUTED CONCENTRATE

During the 6-hour dermal exposure to the undiluted concentrate of Cyantraniliprole 100 g/L OD, only a negligible amount of the applied dose was absorbed (0.36%) (Table 2 & Figure

1). At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (88.6%), while removal of the stratum corneum by tape stripping accounted for approximately 3.43%. Given the amount of dose absorbed (0.36%) and the dose remaining in the tape-stripped skin (0.36%), the total absorbable dose at 6 hours was calculated to be 0.72%. Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.36%, which suggests that only a small portion of bound skin residue associated with the stratum corneum (approximately 0.64% of 3.43%) was absorbed systemically.

Table 2

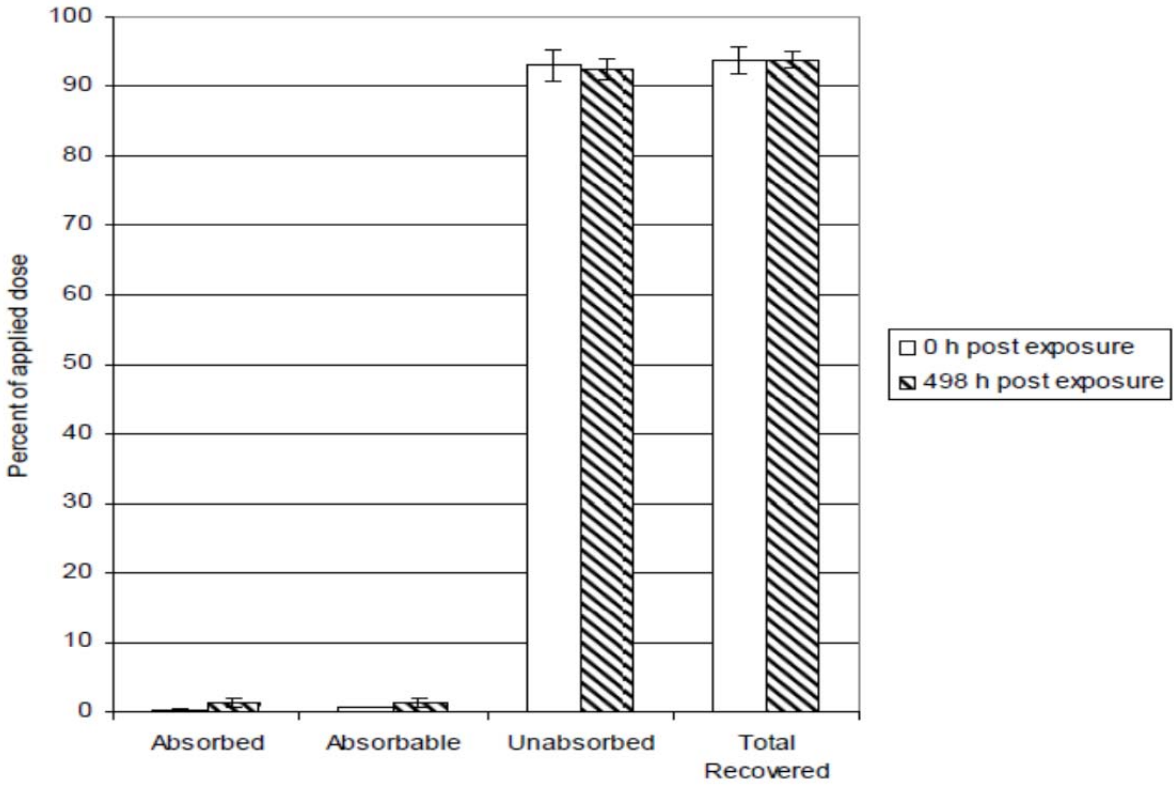
Absorption of radiolabeled cyantraniliprole by rats exposed to Cyantraniliprole 100 g/L OD undiluted concentrate (6-hour exposure) (Treatment group A)

Absorption:	Mean percent of administered radioactivity (%)	
	0 h post exposure	**498 h post exposure**
Unabsorbed dose:		
Skin washes	88.6	87.2
Tape strips (stratum corneum)	3.43	0.79
Protective device[a]	1.01	4.54
Total unabsorbed dose:	93.0	93.8
Absorbed dose:		
Urine	0.04	0.37
Faeces	0.01	0.66
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.30	0.36
Cagewash	ND	0.12
Total absorbed dose:	0.36	1.34
Absorbable dose:		
Total absorbed dose	0.36	1.34
Tape stripped skin	0.36	0.02
Total absorbable dose:	0.72	1.36
Total recovered (material balance):	93.7	93.8

[a] Includes O-ring spacers, rigid mesh covering, and Coban™ body wrap

ND Not detected Data excerpted from Table 3, page 30 of the study report.

Figure 1. Distribution of the recovered dose for 100g cyantraniliprole/L undiluted concentrate (6-hour exposure) (figure excerpted from page 39 of the study report)



C. TREATMENT GROUP B -HGW86 100 G/L OD, 1 G/L AQUEOUS DILUTION

Over the course of a 6-hour dermal exposure to the 1 g/L aqueous dilution of the Cyantraniliprole 100 g/L OD formulation, only a negligible amount of the applied dose was absorbed (0.18%) (Table 3 & figure 2). At the end of the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>86%), while removal the stratum corneum by tape stripping accounted for 4.44%. Given the amount absorbed (0.18%) and the dose remaining in the tape-stripped skin (0.30%), the total absorbable dose at 6 hours was calculated to be 0.47%. Based on a 498-hour recovery/ collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.74%, which was slightly greater, yet comparable to that observed at 6 hours post dose, which suggests that only a small portion of bound skin residue associated with the stratum corneum (approximately 0.27% of 4.44%) was absorbed systemically.

Table 3. Absorption of radiolabeled Cyantraniliprole by rats exposed to Cyantraniliprole 100 g/L OD, 1 g/L aqueous dilution (6-hour exposure) (Treatment group B)

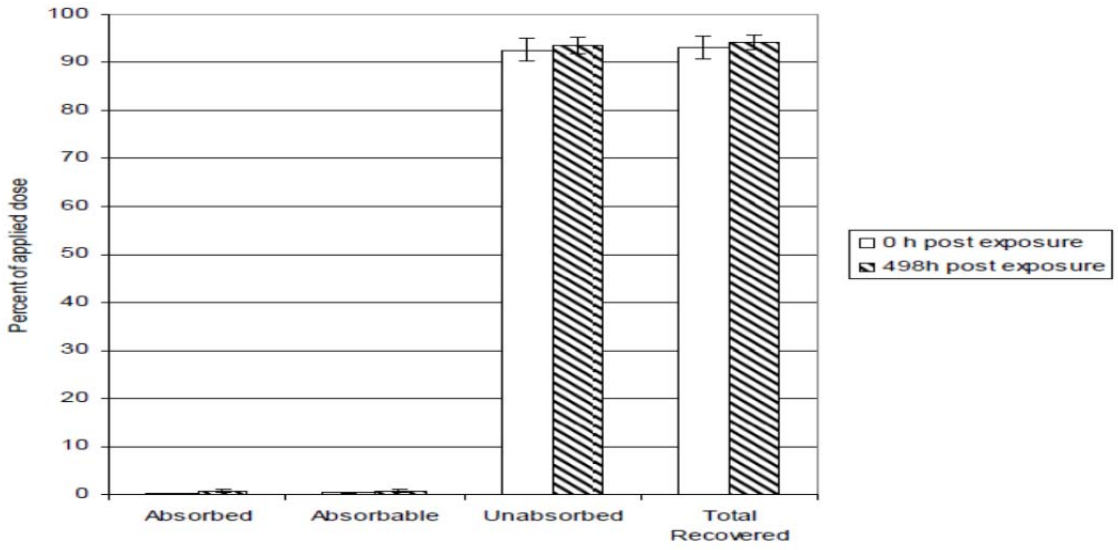
Absorption:	Mean percent of administered radioactivity (%)	
	0 h post exposure	**498 h post exposure**
Unabsorbed dose:		
Skin washes	86.6	89.8
Tape strips (stratum corneum)	4.44	1.57
Protective device[a]	1.57	2.13
Total unabsorbed dose:	92.6	93.5
Absorbed dose:		
Urine	0.02	0.24
Faeces	0.01	0.48
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.19	ND
Cagewash	ND	ND
Total absorbed dose:	0.18	0.72
Absorbable dose:		
Total absorbed dose	0.18	0.72
Tape stripped skin	0.30	0.02
Total absorbable dose:	**0.47**	**0.74**
Total recovered (material balance):	92.6	93.5

[a] Includes rigid mesh covering, O-ring spacers and Coban™ body wrap

ND Not detected Data excerpted from Table 5, page 32 of the study report.

Figure 2 (figure excerpted from page 40 of the study report)

Distribution of recovered dose, 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 100 g/L OD formulation



III. CONCLUSIONS

The dermal absorption of cyantraniliprole formulated as Cyantraniliprole 100 g/L OD was evaluated under *in vivo* conditions in the rat with the undiluted formulation Cyantraniliprole 100 g/L OD concentrate and with a 1 g/L aqueous dilution. The test animals were exposed to the test material for 6 hours. The study report stated that the formulation concentrations and application rates were designed to mimic potential field-use exposures. In addition, the 1g/L aqueous dilution was the lowest concentration that could be prepared and yet provide a reliable quantitative assessment of absorption based on the specific activity of the radiolabelled cyantranilipro used. The results showed that the total absorption was low, and maximum absorption, represented as a percent of the applied dose, was slightly higher for the neat undiluted formulation (0.72-1.36%) than for the aqueous dilution (0.47-0.74%) as summarized below.

Dose formulation	**Total absorbable dose at end of 6-hour exposure (0-h post exposure)**	**Maximum total absorbable dose following 498 hour recovery/collection period**
Undiluted concentrate	0.72	1.36
Aqueous solution	0.47	0.74

The dermal absorption data from both *in vivo* and *in vitro* dermal absorption studies were used to estimate the *in vivo* dermal absorption factorfor human. **The estimated human dermal absorption factors are 0.06% and 0.03% for undiluted concentrate and aqueous dilution, respectively (Table 4).**

Table 4. Summary of dermal absorption of Cyantraniliprole 100 g/L OD

Study	**Sample time**	**Formulation concentrate**	**Aqueous solution**	**Reference**
		% Absorbed	**% Absorbed**	
In vitro - rat skin	6h	10.0	13.9	MRID: 48120210 DuPont-24504
	24 h	10.5	22.7	
In vitro - human skin	6 h	0.24	1.0	
	24 h	0.50	0.92	
In vivo - rat	6 h	0.72	0.47	MRID 48120209 DuPont-24503,
	504 h	1.36	0.74	
Human Dermal Absorption Factor[a]		0.06%	0.03%	

[a] human dermal absorption factor (% absorbed) values were estimated using the formula:
In vivo human absorption (% absorbed) ≅ (*in vitro* human % absorption × *in vivo* rat % absorbed)/ *in vitro* rat % absorption

Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6.2 Comparative dermal absorption, *in vitro* using rat and human skin

Report: Fasano, W.J. (2008); Cyantraniliprole (DPX-HGW86) 100 g/L OD: *In vitro* kinetics of cyantraniliprole in rat and human skin. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-24504. December 17, 2008. MRID 48120210. Unpublished.

Guidelines: OECD 427 (2004), OECD 28 (2004), ECC Sanco/222/2000 Rev.7 (2004)
Deviations: None

GLP: Yes. Signed statements of GLP Compliance, Data Confidentiality, and Quality Assurance were submitted in the study report.

Executive summary:

The dermal penetration of cyantraniliprole 100g/L oil dispersion (OD) formulation was tested in vitro using rat and human skin mounted in a static diffusion cell model with an exposure area of 0.64 cm² maintained at 32ºC (MRID 48120210) . The test substance, Cyantraniliprole 100 g/L OD, was applied as the undiluted OD at 100 g cyantraniliprole/L and as a 1 g cyantraniliprole/L OD aqueous dilution. Penetration and absorption were followed using [^{14}C]-cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied at a rate of 10 µL/cm² to 2 groups of 6 skins per dose level per species. The amount of cyantraniliprole applied per area of skin was approximately 1000 µg/cm² and 10 µg/cm² for the 100 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. Serial receptor fluid samples (2×50 µL) were collected at 0.5, 1, 2, 4, 6 hours (end of exposure) for all replicates, and a final sample was taken at 24 hours for skins held post-exposure. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume. At 6 hours, the skin surfaces of all groups were washed, and one group of 6 skins per dose level per species was terminated (0-hours post-exposure) to determine the distribution of the applied dose at the end of the exposure phase. The remaining 6 skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

For the undiluted concentrate, over the 6-hour exposure phase, total penetration, receptor fluid only, represented 6.30% of the applied dose for rat skin; radiolabeled cyantraniliprole did not penetrate through human skin into the receptor fluid. The penetration rate was 8.91 µg equiv/cm²/h during the initial 6-hour exposure period. By the end of the 6-hour exposure period (0 hours post-exposure), the cumulative amount penetrated per area was 49.7 µg equiv/cm². Washing of the skin at 6 hours removed >77% of the applied dose exclusive of species, which represented a majority of the unabsorbed dose. At the end of the 6-hour exposure period, tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals via the skin, removed 7.17% and 2.18% of the applied dose from rat and human skin,

respectively. The total absorbable dose (receptor fluid plus tape-stripped skin) was approximately 42-fold greater for rat skin (10.0%) than for human skin (0.24%). During the post-exposure period, a portion of the dose contained in the skin continued to partition into the receptor fluid but at a reduced rate compared to the rate of penetration during the 6-hour exposure. T**he maximum absorbable dose was 10.5% and 0.25% for rat and human skin, respectively.**

For 1 g cyantraniliprole/L aqueous dilution, total penetration (receptor fluid only) represented 5.65% of the applied dose for rat skin during the 6-hour exposure period. Cyantraniliprole did not penetrate human skin during the initial 6-hour exposure. The penetration rate for [^{14}C]-cyantraniliprole through the rat skin was negligible (0.08 µg equiv/cm²/h). Cumulative penetration (amount per area) at the end of the exposure was measurable for rat skin only (0.43 µg equiv/cm²). Washing of the skin at the end of the 6 hour exposure removed slightly more of the applied dose from human skin (81.2%) than from rat skin (61.9%). Tape-stripping of the stratum corneum, removed 23.2% and 12.1% of the applied dose from rat and human skin, respectively. The total absorbable dose (receptor fluid plus tape-stripped skin) was approximately 14-fold greater for rat skin (13.9%) than for human skin (1.00%). During the Post-exposure period, a portion of the dose contained in the skin continued to partition into the receptor fluid but at a reduced rate compared to that during the 6-hour exposure period. **The maximum absorbable dose was 20.2% and 0.86% of the applied dose for rat and human skin, respectively.**

The results demonstrate that penetration of cyantraniliprole from the Cyantraniliprole 100 g/L OD formulation, when applied either as the undiluted concentrate or an aqueous dilution was greater for rat skin compared to human skin. **The *in vitro* dermal absorption data on isolated rat and human skin in combination with the *in vivo* rat dermal absorption data weree used to estimate the *in vivo* human dermal absorption factor; the factors were 0.06% for cyantraniliprole 100 g/L OD formulation concentrate and 0.03% for 1g/L aqueous dilution of the concentrate.**

This study is reliable (Acceptable) and fulfilled the OECD guideline (OECD 428 (2004)). *However, the study report should provide the information for the location where the human and rat skin was derived (i.e. abdominal skin).*

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 100 g/L OD
	Lot/Batch #:	HGW86-329
	Purity:	100 g ai/L (undiluted concentrate) 1 g ai/L (field application rate)
	Description:	Liquid
	CAS #:	None for the test formulation 736994-63-1 for the active ingredient
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantraniliprole technical
	Lot/Batch #:	[pyrazole carbonyl-^{14}C[-cyantraniliprole abbreviated as [PC-^{14}C]-cyantraniliprole, Lot# 3562-042
	Radiochemical purity:	[PC-^{14}C]-cyantraniliprole: radiochemical purity >98%
	Specific activity:	[PC-^{14}C]-cyantraniliprole: 44.06 µCi/mg
	Description:	Not specified
	Stability of test compound:	The test material was stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of cyantraniliprole, was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated 100 g/L OD formulation. The blank formulation was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water was used for the field application formulation
5.	Rat skin:	Skin used was from male rats of the Sprague-Dawley strain, Crl:CD®(SD)IGS BR, approximately 6-8 weeks of age. Rats were sacrificed by carbon dioxide asphyxiation and the fur from the dorsal region was carefully shaved using clippers. Any animals showing obvious abrasion within the region of the test skin area were considered unsuitable and discarded. The shaved area was excised, held briefly on wet ice, and then frozen at approximately -20°C until processed.
6.	Human skin:	Samples of human skin from the National Disease Research Interchange, Philadelphia, PA, were stored frozen at approximately 20°C until prepared for use.
7.	Test substance concentrations:	See Table 1

Table 1
Summary of the formulation, target concentration and skin dose

Formulation	Target concentration	Target skin dose
Cyantraniliprole (concentrate)	100 g ai/L	1000 µg ai/cm^2
Cyantraniliprole (aqueous dilution)	1 g ai/L	10 µg ai/cm^2

B. STUDY DESIGN AND METHODS

1. Experimental start and completion dates

   September 02, 2008 to September 12, 2008

2. Dermal penetration and absorption assay

   The dermal penetration and absorption of cyantraniliprole was measured *in vitro* through rat and human skin. Frozen samples of rat and human skin were thawed and full thickness skin was dermatomed to approximately 450 µm. Each skin membrane was mounted over the receptor chamber of a glass in vitro static diffusion cell (see figure in Attachment A) with the stratum corneum uppermost. The receptor chamber was then filled with 0.9% saline and maintained at approximately 32°C for the duration of the experiment. During the exposure phase, the contents of the receptor compartment were continuously stirred using a magnetic flea. The integrity of each membrane was assessed by measurement of electrical impedance prior to application of test substance. Membranes with an impedance of ≥6 k-ohms (rat) and ≥17 k-ohms (human) were considered intact and retained for use on study. Following overnight skin membrane equilibration, the receptor fluid was replaced with 50% (v/v) ethanol in water and the test formulations were applied via the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

   The test substance, cyantraniliprole, was applied as the undiluted concentrate at 100 g ai/L and as a 1 g ai/L aqueous dilution. Penetration and absorption were followed using [^{14}C[-cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied at a rate of 10 µL/cm² to two groups of 6 skins per dose level per species. The amount of cyantraniliprole applied per area of skin was approximately 2000 µg/cm² and 10 µg/cm² for the 200 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. At 6 hours, the skin surface of all groups was washed, and one group of 6 skins per dose level per species was terminated to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining 6 skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

II. RESULTS AND DISCUSSION

A. 100 g/L CYANTRANILIPROLE/L UNDILUTED CONCENTRATE

The penetration rate for [^{14}C]-cyantraniliprole from the Cyantraniliprole 100 g/L OD undiluted concentrate (100 g cyantraniliprole/L) during the initial 6-hour exposure was only measurable for rat skin (8.91 µg equiv/cm²/h) (Table 2 and Figure 1); by the end of the 6-hour exposure period (0 hours post-exposure), the cumulative amount penetrated per area was 49.7 µg equiv/cm² for rat skin. Total penetration, receptor fluid only, represented 6.30% of the applied dose for rat skin (Table 3). Radiolabeled cyantraniliprole from the Cyantraniliprole 100 g/L OD undiluted concentrate did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (Figure 1). Washing of the skin at 6 hours removed >77% of the applied dose exclusive of species, which represented a majority of the unabsorbed dose. At the end of the 6-hour exposure period, tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals via the skin, removed 7.17% and 2.18% of the applied dose from rat and human skin, respectively. The total absorbable dose (receptor fluid plus tape-stripped skin) was approximately 42-fold greater for rat skin (10.0%) than for human skin (0.24%).

For the Post-exposure peiod, a portion of the dose contained in the skin continued to partition into the receptor fluid but at a reduced rate compared to that during the 6-hour exposure; the maximum absorbable dose at 24 hours was 10.5% and 0.25% for rat and human skin, respectively (Table 4 & 5, Figure 1).

Table 2
Penetration kinetics of [^{14}C[-cyantraniliprole from cyantraniliprole 100 g/L OD, 100 g cyantraniliprole/L undiluted concentrate, 0-6 hours (0-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	1.70 ±NA	Radiolabeled cyantraniliprole did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (see Figure 1).
1	3.71±1.95	
2	14.9±7.61	
4	32.2±13.3	
6 (end exposure)	49.7±17.1	
Penetration rate, 0.5-6 hours[a] (µg equiv./cm^2/h)	8.91	NA

[a] Slope of mean data, 0.5-6 hours

Data excerpted from Table 2, page 34 of the study report.

NA = not applicable

Table 3. Recovery of total radioactivity at 6 hours following a 6-hour topical exposure to a 100 g cyantraniliprole/L undiluted concentrate of cyantraniliprole 100 g/L OD (0-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	6.30±2.12	NA
Total absorbed	6.30±2.12	NA
Absorbable dose		
Receptor fluid	6.30±2.12	NA
Tape-stripped skin	3.74±1.92	0.24±0.11
Total absorbable	**10.0±3.23**	**0.24±0.11**
Unabsorbed dose		
Skin wash	77.8±3.93	88.5±4.69
Donor chamber	0.66±0.33	0.79±0.68
Tape strips	7.17±3.81	2.18±1.65
Total unabsorbed	85.6±3.56	91.5±3.24
Total recovered	95.6±2.34	92.0±3.11

NA = not applicable

Data excerpted from Table 3, page 35 of the study report.

Table 4
Penetration kinetics of [^{14}C[-cyantraniliprole from cyantraniliprole 100 g/L OD, 100 g cyantraniliprole/L undiluted concentrate, 0-24 hours (18-hour post-exposure group)

Time (hour)	**Data expressed in cumulative μg equiv./cm^2**	
	Rat	**Human**
0.5	NA	Radiolabeled cyantraniliprole did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (see Figure1).
1	3.31±1.40	
2	11.8 ±3.78	
4	29.3±8.49	
6 (end exposure)	44.1±12.5	
24 (18 hours post-exposure)	67.7±18.2	2.21±NA
Penetration rate, 0.5-6 hours[a]	8.22	NA
Penetration rate, 6-24 hours[b] (μg equiv./cm^2/h)	0.77	NA

[a] Slope of mean data, 0.5-6 hours
[b] Slope of mean data, 6-24 hours

NA = not applicable

Data excerpted from Table 5, page 37 of the study report.

Figure 1
Penetration kinetics of [14C]Cyantraniliprole from Cyantraniliprole 100 g/L OD, 100 g cyantraniliprole/L undiluted concentrate, 0-24 hours (18-hour post-exposure group)
(Figure excerpted from Figure 9, page 57 of the report)

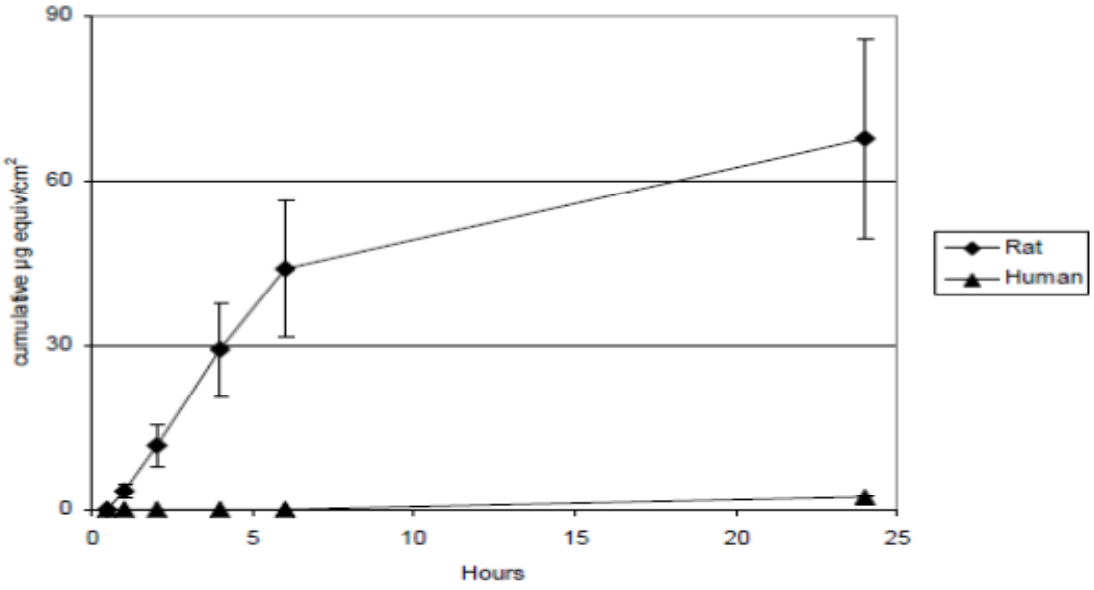


Table 5. Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 100 g cyantraniliprole /L undiluted concentrate of cyantraniliprole 100 g/L OD (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	8.71±2.31	0.30±NA
Total absorbed	8.71±2.31	0.30±NA
Absorbable dose		
Receptor fluid	8.71±2.31	0.30±NA
Tape-stripped skin	1.82±1.15	0.20±0.09
Total absorbable	**10.5±2.34**	**0.50±0.15**
Unabsorbed dose		
Skin wash	81.9±4.72	92.2±1.53
Donor chamber	0.31±0.17	0.45±0.29
Tape strips	3.63±2.92	1.87±0.88
Total unabsorbed	86.1±2.89	94.5±2.20
Total recovered	96.6±1.98	94.8±2.31

NA= not applicable Data excerpted from Table 6, page 38 of the study report.

B. CYANTRANILIPROLE, 1 G CYANTRANILIPROLE/L AQUEOUS DILUTION

The penetration rate for [^{14}C]-cyantraniliprole during the 6-hour exposure period from a 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 100 g/L OD formulation was negligible and only measurable for rat skin (0.08 µg equiv/cm²/h) (Table 6 & Figure 2).

Cumulative penetration (amount per area) at the end of the exposure was measurable for rat skin only (0.43 µg equiv/cm²); radiolabeled cyantraniliprole did not penetrate human skin during the initial 6-hour exposure (Figure 2). Total penetration (receptor fluid only) represented 5.65% of the applied dose for rat skin (Table 7). Washing of the skin at the end of the 6 hour exposure removed the slightly more of the applied dose from human skin (81.2%) than from rat skin (61.9%). Tape-stripping of the stratum corneum removed 23.2% and 12.1% of the applied dose from rat and human skin, respectively. The total absorbable dose (receptor fluid plus tape-stripped skin) was approximately 14-fold greater for rat skin (13.9%) than for human skin (1.00%).

For the Post-exposure peiod, a portion of the dose contained in the skin continued to partition into the receptor fluid but at a reduced rate compared to that during the 6-hour exposure (Table 8 & Figure2); the maximum absorbable dose at 24 hours was 22.72% and 0.92% for rat and human skin, respectively (Table 9).

Table 6
Penetration kinetics of [^{14}C[-cyantraniliprole from cyantraniliprole 100 g/L OD, 1 g cyantraniliprole/L aqueous dilution, 0-6 hours (0-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	NA	Radiolabeled cyantraniliprole did not penetrate human skin during the initial 6-hour exposure (see Figure 2).
1	0.04±0.01	
2	0.10±0.03	
4	0.27±0.10	
6 (end exposure)	0.43±0.18	
Penetration rate, 0.5-6 hours[a] (µg equiv./cm^2/h)	0.08	NA

[a] Slope of mean data, 0.5-6 hours
ND = not detected
NA = not applicable

Data excerpted from Table 9, page 41 of the study report.

Table 7
Recovery of total radioactivity at 6 hours following a 6-hour topical exposure to a 1 g cyantraniliprole/L aqueous dilution of cyantraniliprole 100 g/L OD (0-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	5.65±2.34	NA
Total absorbed	5.65±2.34	NA
Absorbable dose		
Receptor fluid	5.65±2.34	NA
Tape-stripped skin	8.27±8.53	1.00±1.07
Total absorbable	**13.9±8.77**	**1.00±1.07**
Unabsorbed dose		
Skin wash	61.9±7.79	81.2±12.8
Donor chamber	0.27±0.05	1.13±0.86
Tape strips	23.2±8.07	12.1±8.57
Total unabsorbed	85.6±7.68	94.5±7.55
Total recovered	99.5±2.60	95.5±7.47

NA = not applicable

Data excerpted form Table 10, page 42 of the study report.

Table 8
Penetration kinetics of [^{14}C[-cyantraniliprole from cyantraniliprole 100 g/L OD, 1 g cyantraniliprole/L aqueous dilution, 0-24 hours (18-hour post-exposure group)

Time (hour)	**Data expressed in cumulative μg equiv./cm^2**	
	Rat	**Human**
0.5	0.10±0.08	Radiolabeled cyantraniliprole did not penetrate human skin during the initial 6-hour exposure.
1	0.16±0.08	
2	0.16±0.13	
4	0.31±0.22	
6 (end exposure)	0.42±0.31	
24 (18 hours post-exposure)	0.81±0.43	0.04±0.02
Penetration rate, 0.5-6 hours[a]	0.06	NA
Penetration rate, 6-24 hours[b]	0.02	NA
(μg equiv./cm^2/h)		

[a] Slope of mean data, 0.5-6 hours

[b] Slope of mean data, 6-24 hours

NA = not applicable

Data excerpted from Table 12, page 44 of the study report.

Figure 2
Penetration kinetics of [14C]Cyantraniliprole from Cyantraniliprole 100 g/L OD, 1 g cyantraniliprole/L aqueous dilution, 0-24 hours (18-hour post-exposure group)
(Figure excerpted from Figure 15, page 63 of the report)

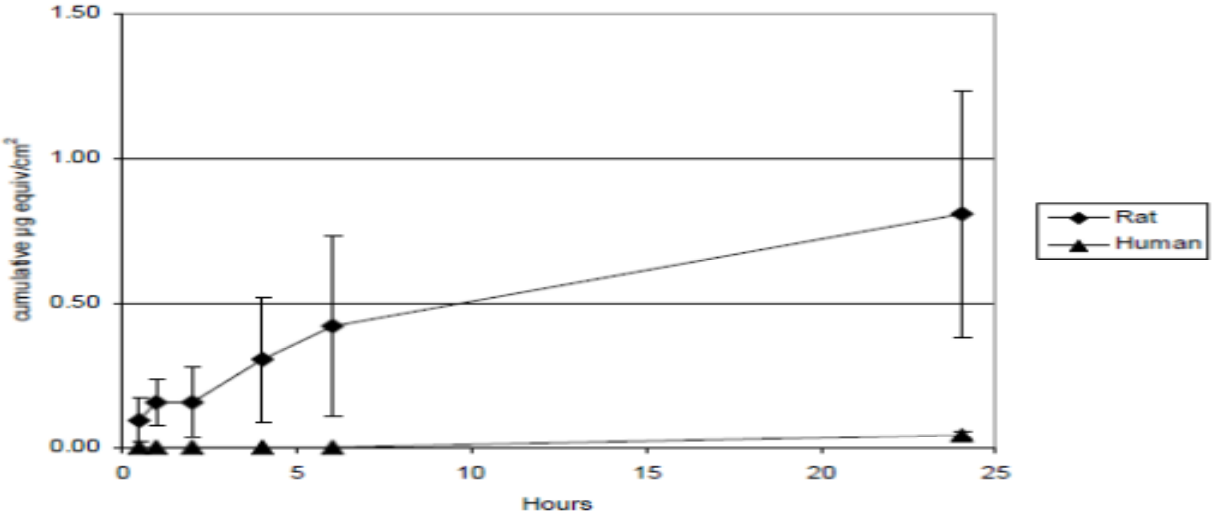


Table 9
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 1 g cyantraniliprole ai/L aqueous dilution of cyantraniliprole 100 g/L OD (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	10.7±5.49	0.61±0.24
Total absorbed	10.7±5.49	0.61±0.24
Absorbable dose		
Receptor fluid	10.7±5.49	0.61±0.24
Tape-stripped skin	12.0±1.12	0.31±0.20
Total absorbable	**22.7±5.80**	**0.92±0.42**
Unabsorbed dose		
Skin wash	52.7±9.3	73.3±2.61
Donor chamber	0.16±0.16	1.37±1.22
Tape strips	18.8±7.11	10.37±7.18
Total unabsorbed	76.3±1.15	80.9±5.68
Total recovered	94.7±4.49	82.3±5.71

Data excerpted from Table 13, page 45 of the study report.

III. CONCLUSIONS

The results obtained in this study, using an *in vitro* dermal static diffusion cell model, demonstrate that penetration and absorption of cyantraniliprole from the Cyantraniliprole 100

g/L OD formulation, when applied either as the undiluted concentrate or an aqueous dilution was greater for rat skin compared to human skin. During the 6-hour exposure period, cyantraniliprole did not penetrate through the isolated human skin. The total absorbable doses at 24 hours, for undiluted concentrate were 10.5% and 0.5% for the formulation concentrate for rat and human, respectively; for the aqueous dilution the values were 22.7% and o.92% for rat and human skin, respectively. A summary of the dermal absorption is presented in Table 10 which includes the value of *in vivo* dermal absorption in rats (MRID 48120209). From these data, a human dermal absorption factor can be estimated. The extrapolated dermal absorption for cyantraniliprole 100 g/L OD are based on the maximum *in vivo* absorption in rats 504 hours following initiation of exposure and the comparative absorption *in vitro* in rat and human skin 24 hours post-exposure. Since the 24-hour *in vivo* dermal absorption value for the rat was not available, the 504 hour value (maximum value) was used. **The estimated human dermal absorption factors are 0.06% and 0.03% for undiluted concentrate and aqueous dilution, respectively.**

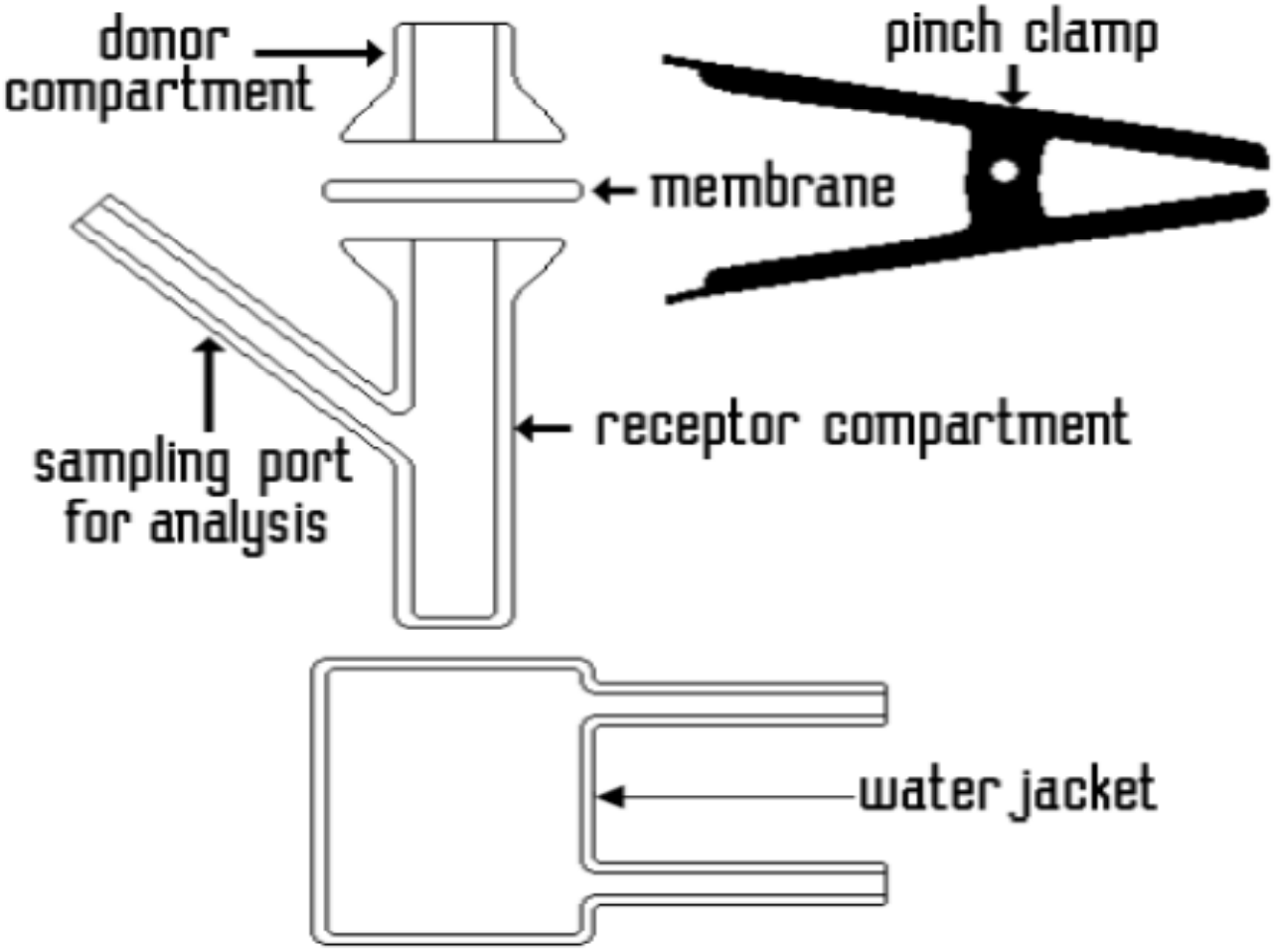
Table 10
Summary of dermal absorption of Cyantraniliprole 100 g/L OD

Study	Sample time	Formulation concentrate	Aqueous solution	Reference
		% Absorbed	% Absorbed	
In vitro - rat skin	6h	10.0	13.9	MRID: 48120210 DuPont-24504
	24 h	10.5	22.7	
In vitro - human skin	6 h	0.24	1.0	
	24 h	0.50	0.92	
In vivo - rat	6 h	0.72	0.47	MRID 48120209 DuPont-24503,
	504 h	1.36	0.74	
Human Dermal Absorption Factor[a]		0.06%	0.03%	

[a] human dermal absorption factor (% absorbed) values were estimated using the formula:
In vivo human absorption (% absorbed) ≅ (*in vitro* human % absorption × *in vivo* rat % absorbed)/ *in vitro* rat % absorption

Attachment A

Static diffusion cell



Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6 Dermal absorption

IIIA 7.6.1 Dermal absorption, *in vivo* in the rat

Report: Fasano, W.J. (2009); Cyantraniliprole (DPX-HGW86) 200 g/Liter SC: *In vivo* dermal absorption of cyantraniliprole in the rat. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-24501. February 16, 2009. MRID 48120313. Unpublished.

Guidelines: OECD 427 and No.28 (2004)
SANCO/222/2000 rev.7 (2004)
Deviations: None

GLP: Yes. Signed statements of GLP, Data Confidentiality, and Quality Assurance were included in the report.

Executive summary:

The cyantraniliprole formulated as Cyantraniliprole 200 g/L SC was investigated under *in vivo* conditions in a dermal absorption study with Crl:CD®(SD)IGS BR (8 males/group) (MRID 48120313). Cyantraniliprole 200 g/L SC, was applied as the undiluted concentrate at 200 g cyantraniliprole/L and as a 1 g cyantraniliprole/L aqueous dilution. Absorption was followed using [^{14}C]-cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied to a 10.5 cm² shaved area on the dorso-lumbar region at a rate of 10 µL/cm² to 2 groups of 4 rats per dose level. The amount of cyantraniliprole applied per area of skin was approximately 2000 µg/cm² and 10 µg/cm² for the 200 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively . The applied formulation remained in contact with the skin for 6 hours. At 6 hours, the skin surface of all rats was washed with a dilute soap solution and one group of 4 rats per dose concentration was sacrificed to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining 4 rats at each dose level were maintained until 504 hours post-dose (498 hours post-exposure; 21 days). At sacrifice, the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

For the undiluted concentrate, only a minor amount of the applied dose was absorbed (0.33%) during the 6-hour dermal exposure. At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (>87%), while removal of the stratum corneum by tape stripping accounted for approximately 2.19%. Given the amount of dose absorbed (0.33%) and the dose remaining in the tape-stripped skin (0.12%), the total absorbable dose at 6 hours was calculated to be 0.45%. **Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.34% of the applied dose.**

For the 1 g/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation, over the course of a 6-hour dermal exposure to the only a minor amount of the applied dose was absorbed

(0.47%). At the end of the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>87%), while removal the stratum corneum by tape stripping accounted for 4.45%. Given the amount absorbed (0.47%) and the dose remaining in the tape-stripped skin (0.36%), the total absorbable dose at 6 hours was calculated to be 0.83%. **Based on a 498-hour recovery/collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.93% of the applied dose.**

In summary, following a 6-hour dermal exposure to Cyantraniliprole 200 g/L SC formulation, when applied either as the undiluted concentrate or as an aqueous dilution, total absorption was low, and maximum absorption was higher for the aqueous dilution (0.93%) than for neat undiluted formulation (0.34%). The dermal absorption data from both *in vivo* and *in vitro* dermal absorption studies were used to estimate the *in vivo* human dermal absorption factor. The estimated factors were 0.02% for cyantraniliprole 200 g/L SC formulation concentrate and 0.43% for aqueous dilution.

This study is reliable (acceptable) and meets the requirements for an *in vivo* dermal absorption study (OECD 427 and 428 (2004)).

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 200 g/L SC
	Lot/Batch #:	HGW86-308
	Purity:	200 g ai/L (undiluted concentrate) 1 g ai/L (field application rate)
	Description:	Soluble Concentrate
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantraniliprole
	Lot/Batch #:	[pyrazole carbonyl-^{14}C]-cyantraniliprole abbreviated as [PC-^{14}C]-cyantraniliprole, Lot#: 3562-042
	Radiochemical purity:	[PC-^{14}C]-cyantraniliprole: radiochemical purity >98%
	Specific activity:	[PC-^{14}C]-cyantraniliprole: 44.06 μCi/mg
	Description:	Not specified in report
	Stability of test compound:	The test materials were stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of Cyantraniliprole, was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated formulation. The blank formulation was stored at room temperature.

4. Vehicle and/or positive control:	Water for the aqueous dilution
5. Test animals	
Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Sex	Males
Age at dosing:	Approximately 6-8 weeks old
Weight at dosing:	216-230 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	6 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in all-glass metabolism units
6. Environmental conditions	
Temperature:	22-24°C
Humidity:	40-60%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   November 12, 2008 to December 10, 2008

2. Animal assignment and treatment

   On the day prior to dermal dosing, the back and shoulders of each animal was clipped free of hair and the clipped area washed with a 2% Ivory® Soap solution in water. Following shaving and washing, three O-rings (one stacked upon the other) with an internal area of approximately 10.5 cm2, were glued to the clipped area on the back using Instant Krazy Glue® Gel. The O-rings were then covered with Coban™ body wrap to prevent contamination

   Doses applied and target parameters are summarized in Table 1.

Table 1
Study design: Summary of doses applied and target parameters for the *in vivo* assessment of dermal absorption of Cyantraniliprole in the rat

Group	Dose concentration of Cyantraniliprole	Skin dose level of Cyantraniliprole	Number of animals[a]	µCi/rat
A	200 g/L[b]	2000 µg/cm^2	8	8
B	1 g/L[c]	10 µg/cm^2	8	5

[a] Based on 4 rats at 2 post-exposure collection timepoints
[b] Rats in group A were exposed to a single application of the undiluted formulation concentrate.
[c] Rats in group B were exposed to a single application of an aqueous dilution of the formulation.

Two sacrifice time intervals for Group A and Group B treatment groups were included in this study:

Interval I: 4 animals were sacrificed 6 hours after dermal application (0 hours post-exposure).

Interval II: 4 animals were sacrificed 504 hours after dermal application (498 hours post-exposure).

3. Dose formulation and analysis

The Cyantraniliprole 200 g/L SC undiluted concentrate was prepared by mixing radiolabeled cyantraniliprole (5.81 mg) with non-radiolabeled cyantraniliprole (666.14 mg) followed by the addition of formulation blank (2.688 g), and glass beads (5.4 g @ 0.5-0.75 mm). The ingredients were wet-milled at approximately 3000 rpm for approximately 50 minutes. The wet-milled ingredients were separated from the glass beads by syringe and placed into a glass vial.

The Cyantraniliprole 200 g/L SC aqueous dilution was prepared by mixing radiolabeled cyantraniliprole (3.36 mg), formulation blank (13.44 g), deionized water (3.34 g), and glass beads(5.4 g @ 0.5-0.75 mm). The ingredients were wet-milled at approximately 3000 rpm for approximately 50 minutes. The aqueous dilution was separated from the glass beads by syringe and placed into a glass vial.

The formulated products were stored frozen at ≤-10°C.

The homogeneity and amount of radiolabeled cyantraniliprole (µCi/g) in each formulated dose was determined by subjecting aliquots of the prepared dose to radioanalysis by liquid scintillation counting (LSC). The concentration of cyantraniliprole in each dose formulation was determined chromatographically by HPLC-UV. The results of homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled cyantraniliprole (µCi/mg) for the formulated doses. The radiochemical purity of the neat radiolabeled cyantraniliprole

and the stability of radiolabeled cyantraniliprole in the prepared dose formulations were determined by HPLC-radiochromatography.

4. Dosing

On the day of dosing, the protective gauze wrap was removed and the formulated products were applied within the O-ring area at a rate of 10 μL/cm^2. Following dosing, the dose site was protected with a rigid mesh covering and Coban™ body wrap and each animal was separately housed in an all-glass metabolism cage. The applied formulation remained in contact with the skin for 6 hours. After 6 hours, the skin surface of all rats was washed using natural sponge pieces soaked in 2% Ivory® Soap and water.

5. In-life sample collection

Urine and faeces were collected during the 0-6 hour exposure period, at 6-12 hours, 12-24 hours and every 24 hours thereafter until sacrifice. The 6-hour aqueous soap solution washes of the application site, the sponges used during the washing, and the body wrap and rigid mesh covering (from the end of the exposure phase and at sacrifice) were collected for analysis.

6. Sacrifice

Animals were exposed to carbon dioxide asphyxiation and exsanguinated *via* cardiac puncture. The application skin site was excised and tape-stripped to remove the stratum corneum. The skin washes, Coban™ body wrap, cage washes, residual feed, skins from the application site, tape strips, skins from a non-dosed area, blood (whole blood, plasma, and red blood cells), and remaining carcass were analysed for total radioactivity.

7. Sample analysis

Urine, skin washes, cage washes, blood plasma were assayed directly by LSC. The protective mesh cover, Coban™ body wrap, O-ring spacers, and tape strips were extracted using acetonitrile and analysed directly by LSC. Faeces, residual feed, carcasses were homogenised in water and combusted prior to LSC. Skin samples (both the application skin and non-dose skin) and wash sponges pieces were digested in Soluene®-350 and analysed directly by LSC.

8. Statistics

Group data is presented as the mean result.

II. RESULTS AND DISCUSSION

A. RADIOCHEMICAL PURITY, CONCENTRATION AND STORAGE STABILITY

The purity of radiolabeled Cyantraniliprole was >95%. Analyses confirmed that cyantraniliprole was present in the doing formulations at the appropriate concentrations and

with the appropriately amount of radiolabeled test substance. [^{14}C]-cyantraniliprole and the formulations were shown to be stable under the conditions of this study.

B. TREATMENT GROUP A – CYANTRANILIPROLE 200 g/L SC UNDILUTED CONCENTRATE

During the 6-hour dermal exposure to the undiluted concentrate of Cyantraniliprole 200 g/L SC, only a minor amount of the applied dose was absorbed (0.33%). At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (>87%), while removal of the stratum corneum by tape stripping accounted for approximately 2.19%. Given the amount of dose absorbed (0.33%) and the dose remaining in the tape-stripped skin (0.12%), the total absorbable dose at 6 hours was calculated to be 0.45%. Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.34%. (Table)

Table 2
Absorption of radiolabeled cyantraniliprole by rats exposed to Cyantraniliprole 200 g/L SC undiluted concentrate (Treatment group A)

Absorption:	Mean percent of administered radioactivity (%)	
	0 h post exposure	**498 h post exposure**
Unabsorbed dose:		
Skin washes	87.39	90.29
Tape strips (stratum corneum)	2.19	0.023
Protective device[a]	1.159	2.34
Total unabsorbed dose:	90.75	92.65
Absorbed dose:		
Urine	0.007	0.094
Faeces	0.002	0.097
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.277	ND
Cagewash	0.025	0.130
Total absorbed dose:	0.331	0.342
Absorbable dose:		
Total absorbed dose	0.331	0.342
Tape stripped skin	0.122	0.002
Total absorbable dose:	**0.453**	**0.342**
Total recovered (material balance):	91.20	92.99

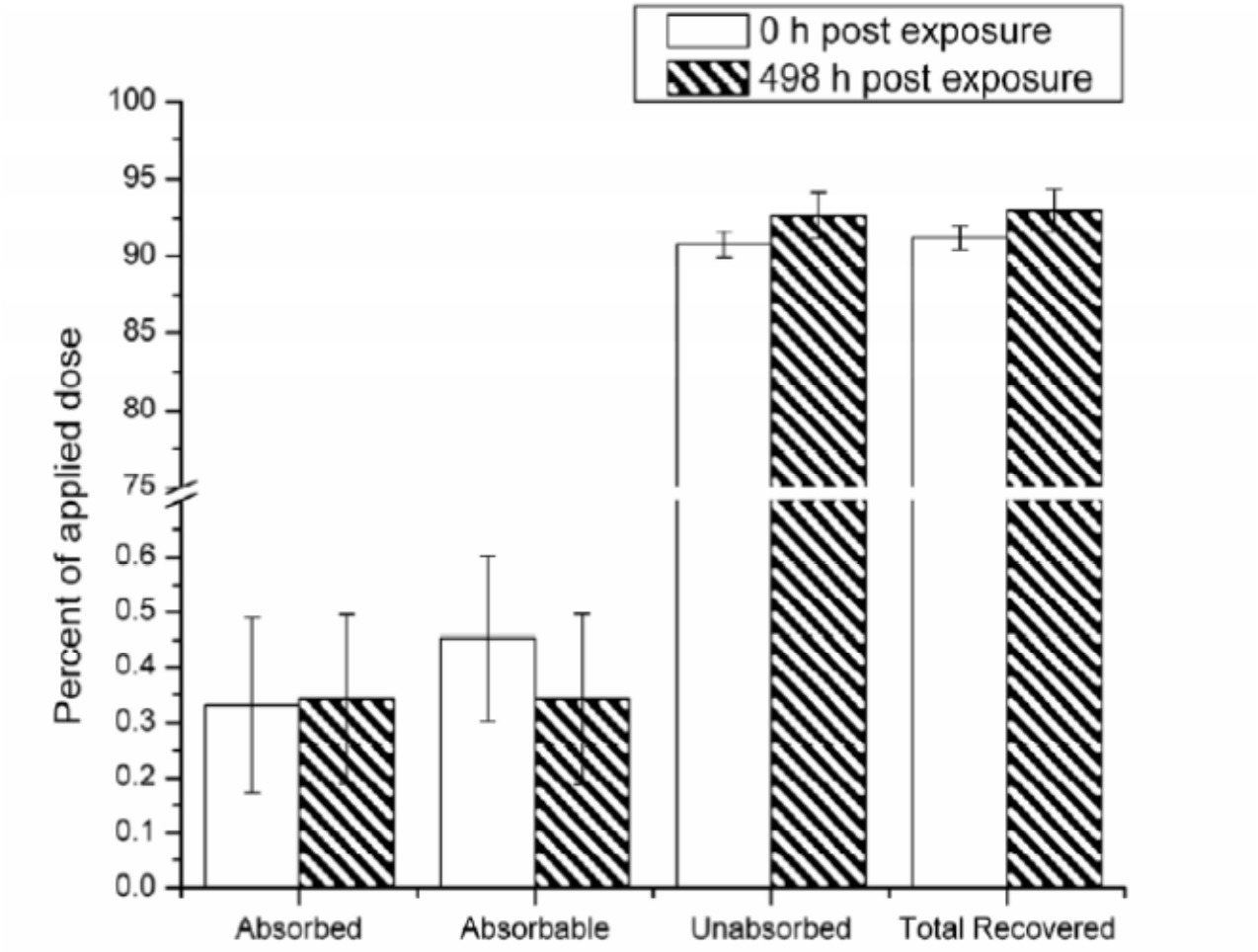
[a] Includes O-ring spacers, rigid mesh covering, and Coban™ body wrap

ND Not detected

Data excerpted from Tabl3 3, page 29 of the report.

Figure 1(Figure excerpted from page 40 of the study report)

Distribution of recovered dose, 200 g cyantraniliprole/L undiluted concentrate of the Cyantraniliprole 200 g/L SC formulation



C. TREATMENT GROUP B -HGW86 200 G/L SC, 1 G/L AQUEOUS DILUTION

Over the course of a 6-hour dermal exposure to the 1 g/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation, only a minor amount of the applied dose was absorbed (0.47%). At the end of the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>87%), while removal the stratum corneum by tape stripping accounted for 4.45%. Given the amount absorbed (0.47%) and the dose remaining in the tape-stripped skin (0.36%), the total absorbable dose at 6 hours was calculated to be 0.83%. Based on a 498-hour recovery/collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 0.93%. (Table)

Table 3
Absorption of radiolabeled Cyantraniliprole by rats exposed to Cyantraniliprole 200 g/L SC, 1 g/L aqueous dilution
(Treatment group B)

Absorption:	Mean percent of administered radioactivity (%)	
	0 h post exposure	**498 h post exposure**
Unabsorbed dose:		
Skin washes	87.34	88.72
Tape strips (stratum corneum)	4.447	0.443
Protective device[a]	1.992	2.988
Total unabsorbed dose:	93.78	92.15
Absorbed dose:		
Urine	0.007	0.204
Faeces	ND	0.450
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.382	ND
Cagewash	0.042	0.271
Total absorbed dose:	0.466	0.925
Absorbable dose:		
Total absorbed dose	0.466	0.925
Tape stripped skin	0.364	0.006
Total absorbable dose:	**0.829**	**0.931**
Total recovered (material balance):	94.61	93.08

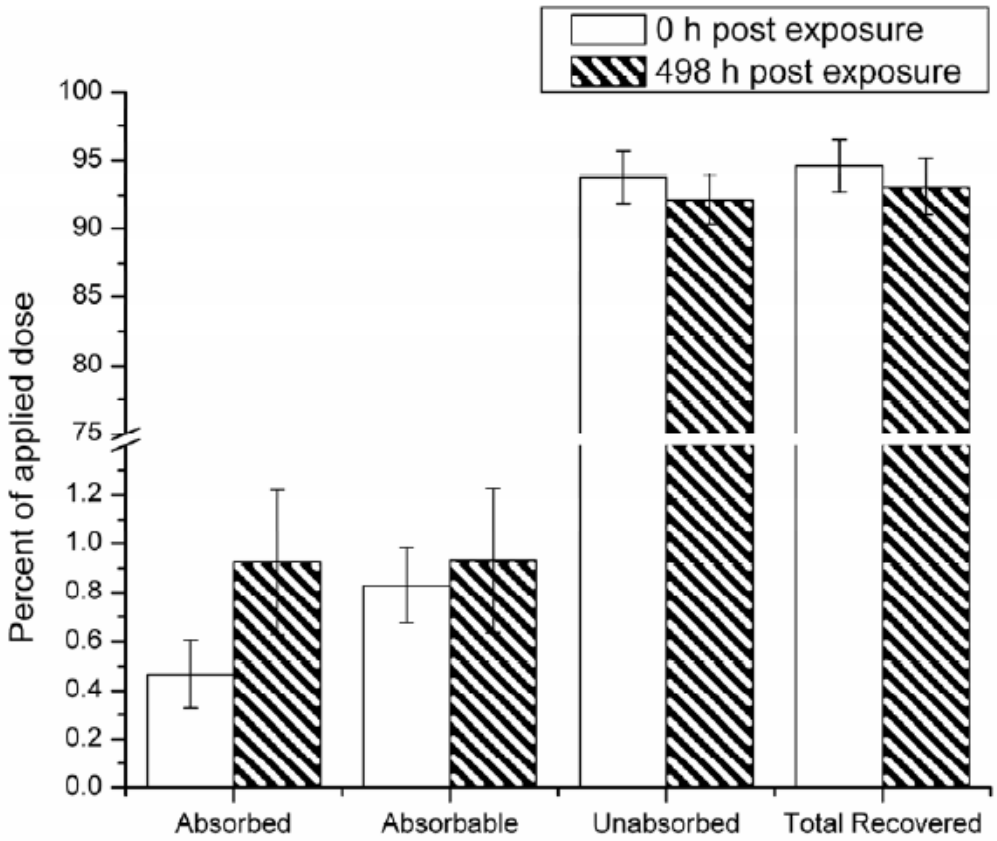
[a] Includes rigid mesh covering, O-ring spacers and Coban™ body wrap

ND Not detected

Data excerpted from Table 5, page 31 of the report.

FIGURE 2 (Figure excerpted from page 41 of the study report)

Distribution of recovered dose, 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation



III. CONCLUSIONS

The dermal absorption of cyantraniliprole formulated as Cyantraniliprole 200 g/L SC was investigated under *in vivo* conditions in the rat. The report stated that the formulation concentrations and application rates were designed to mimic potential field-use exposures. In addition, the 1g/L aqueous dilution was the lowest concentration that could be prepared and yet provide a reliable quantitative assessment of absorption based on the specific activity of the radiolabeled cyantraniliprole used. The results of this study showed that following a 6-hour dermal exposure to Cyantraniliprole 200 g/L SC formulation, either as the undiluted concentrate or as an aqueous dilution, total absorbable dose at different times was summarized below:

	Percent (%) of the Applied Dose	
Dose formulation	**Total absorbable dose at end of 6-hour exposure (0-h post exposure)**	**Maximum total absorbable dose following 498 hour recovery/collection period**
Undiluted concentrate	0.453	0.342
Aqueous solution	0.829	0.931

The human dermal absorption factors were estimated based on the maximum *in vivo* absorption in rats 504 hours following initiation of exposure and the comparative absorption *in vitro* with rat and human skin 24 hours post-exposure (Table 4). Since the 24-hour *in vivo* dermal absorption value for the rat was not available and the % absorbed values for the 6 hr and 504 hr were similar, the 504-hour value (maximum value) was used. The estimated human dermal absorption factors were 0.02% and 0.43% for the concentrate and aqueous dilution, respectively.

Table 4. Cyantraniliprole 200 g/L SC: Dermal absorption of cyantraniliprole

Study	Sample time	Formulation concentrate	Aqueous solution	Reference
		% Absorbed	% Absorbed	
In vitro - rat skin	6h	3.94	10.8	MRID 48120314 DuPont-24502
	24 h	3.69	11.5	
In vitro - human skin	6 h	0.12	4.7	
	24 h	0.24	5.28	
In vivo - rat	6 h	0.453	0.829	MRID 48120313 DuPont-24501
	504 h	0.342	0.931	
Human dermal absorption factor[a]		**0.02**	**0.43**	

[a] Human absorption factor (% absorbed) ≅ (*in vitro* human % absorption × *in vivo* rat % absorbed)/ *in vitro* rat % absorption; since the % absorbed values for the 6 hr and 504 hr are similar for *in vivo* rat dermal absorption study, the values for 504 hour are used.

Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6.2 Comparative dermal absorption, *in vitro* using rat and human skin

Report: Fasano, W.J. (2009); Cyantraniliprole 200 g/Liter SC: *In vitro* kinetics of cyantraniliprole in the rat and human skin. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-24502. February 16, 2009. MRID 48120314. Unpublished.

Guidelines: OECD 28 and 428 (2004)
SANCO/222/2000 rev.7 (2004)
Deviations: None

GLP: Yes. Signed statements of GLP, Data Confidentiality, and Quality Assurance were included in the study report.

Executive summary:

In an *in vitro* dermal absorption study (MRID 48120314), cyantraniliprole 200 g/L SC formulation was evaluated using a static diffusion cell model with isolated rat and human skin (exposure area of 0.64 cm² and maintained at 32°C). The test substance, Cyantraniliprole 200 g/L SC, was applied as the undiluted suspension concentrate (SC) at 200 g cyantraniliprole/L and as a 1 g cyantraniliprole/L aqueous dilution. Penetration and absorption were followed using [^{14}C]-cyantraniliprole, which was uniformly blended into the formulations prior to application. The test articles were applied at a rate of 10 µL/cm² to 2 groups of 6 skins per dose level per species. The amount of cyantraniliprole applied per area of skin was approximately 2000 µg/cm² and 10 µg/cm² for the 200 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. Serial receptor fluid samples (2×50 µL) were collected at 0.5, 1, 2, 4, 6 hours (end of exposure) for all replicates, and a final sample was taken at 24 hours for skins held post-exposure. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume. At 6 hours, the skin surfaces of all groups were washed, and one group of 6 skins per dose level per species was terminated (0-hours post-exposure) to determine the distribution of the applied dose at the end of the exposure phase. The remaining 6 skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

For the cyantraniliprole 200 g/L SC undiluted concentrate, the total penetration, receptor fluid only, represented 1.01% of the applied dose for rat skin over the 6-hour exposure phase, but it did not penetrate through human skin into the receptor fluid. The penetration rate for [^{14}C]-cyantraniliprole during the initial 6-hour exposure was for rat skin was 2.93 µg equiv/cm²/h. By the end of the 6-hour exposure period (0 hours post-exposure), the cumulative amount penetrated per area was for rat skin was 20.6 µg equiv/cm². Washing of the skin at 6 hours removed >89% of the applied dose exclusive of species, which represented a majority of the unabsorbed dose. At the end of the 6-hour exposure period, tape-stripping of the stratum corneum removed 3.73% and 1.09% of the applied dose from rat and human skin, respectively. The total absorbable dose

at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 33-fold greater for rat skin (3.94%) than for human skin (0.12%). At post-exposure, a portion of the dose contained in rat skin continued to partition into the receptor fluid but at a reduced rate compared to that during the 6-hour exposure. **The maximum absorbable dose 18 hours post-exposure (or 24 hours) was 3.69% and 0.24% for rat and human skin, respectively. It should be noted that the maximum absorbable dose for human skin was the amount remained in the tape-stripped skin.**

For 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation, during the 6-hour exposure, the total penetration (receptor fluid only) represented 2.86% and 0.49% of the applied dose for rat and human skin, respectively. The penetration rate for [^{14}C]-cyantraniliprole during the 6-hour exposure period was only measurable for rat skin (0.03 µg equiv/cm²/h). Cumulative penetration (amount per area) at the end of the exposure was 6.5-fold greater for rat skin (0.20 µg equiv/cm²) than for human skin (0.03 µg equiv/cm²). Washing of the skin at the end of the 6 hour exposure removed ≥62% of the applied dose, exclusive of species. Tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via* the skin, removed 22.9% and 22.0% of the applied dose from rat and human skin, respectively; of the total dose retained in the stratum corneum slightly more was contained in tapes 1 and 2 for human skin (~76%) than for rat skin (~56%). The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 2.3-fold greater for rat skin (10.8%) than for human skin (4.70%). During post-exposure period, a portion of the dose contained in rat skin continued to partition into the receptor fluid; radiolabeled cyantraniliprole was not detected in receptor fluid from human skin until the last collection time point (18 hours post-exposure). **The maximum absorbable dose 18 hours post-exposure (or 24 hours) was 11.5% and 5.28% for rat and human skin, respectively.**

The results of this study demonstrate that absorption of cyantraniliprole from the Cyantraniliprole 200 g/L SC formulation, when applied either as the undiluted concentrate or an aqueous dilution was greater for rat skin than human skin. The *in vitro* dermal absorption data on isolated rat and human skin in combination with the *in vivo* rat dermal absorption data were used to estimate the *in vivo* human dermal absorption factor; **the estimated factors were 0.02% for cyantraniliprole 100 g/L SC formulation concentrate and 0.43% for 1g/L aqueous dilution of the concentrate.**

This study is reliable (acceptable) and meet the OECD guideline requirements of an *in vitro* dermal absorption study (OECD 28 and 428 (2004)). *However, the study report should provide the information for the location where the human and rat skin was derived (i.e. abdominal skin).*

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 200 g/L SC
	Lot/Batch #:	HGW86-308
	Purity:	200 g ai/L (undiluted concentrate) 1 g ai/L (field application rate)
	Description:	Soluble concentrate (liquid)
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantraniliprole technical
	Lot/Batch #:	[pyrazole carbonyl-^{14}C]-cyantraniliprole abbreviated as [PC-^{14}C]-cyantraniliprole, Lot # 3562-402
	Radiochemical purity:	[PC-^{14}C]-cyantraniliprole: Radiochemical purity >98%
	Specific activity:	[PC-^{14}C]-cyantraniliprole: 44.06 µCi/mg
	Description:	Not specified
	Stability of test compound:	The test material was stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of cyantraniliprole, was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated Cyantraniliprole 200 g/L SC formulation. The blank formulation was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water was used for the field application formulation
5.	Rat skin:	Skin used was from male rats of the Sprague-Dawley strain, Crl:CD®(SD)IGS BR, approximately 6-8 weeks of age. Rats were sacrificed by carbon dioxide asphyxiation and the fur from the dorsal region was carefully shaved using clippers. Any animals showing obvious abrasion within the region of the test skin area were considered unsuitable and discarded. The shaved area was excised, held briefly on wet ice, and then frozen at approximately -20°C until processed.
6.	Human skin:	Samples of human skin from the National Disease Research Interchange, Philadelphia, PA, were stored frozen at approximately 20°C until prepared for use.
7.	Test substance concentrations:	See Table 1

Table 1
Summary of the formulation, target concentration and skin dose

Formulation	Target concentration	Target skin dose
Cyantraniliprole (concentrate)	200 g ai/L	2000 µg ai/cm^2
Cyantraniliprole (aqueous dilution)	1 g ai/L	10 µg ai/cm^2

B. STUDY DESIGN AND METHODS

1. Study start and completion dates

   November 12, 2008 to November 26, 2008

2. Dermal penetration and absorption assay

   The dermal penetration and absorption of cyantraniliprole was measured *in vitro* through rat and human skin. Frozen samples of rat and human skin were thawed and full thickness skin was dermatomed to approximately 450 µm. Each skin membrane was mounted over the receptor chamber of a glass *in vitro* diffusion cell (Figure shown in Attachment A) with the stratum corneum uppermost. The receptor chamber was then filled with 0.9% saline and maintained at approximately 32°C for the duration of the experiment. During the exposure phase, the contents of the receptor compartment were continuously stirred using a magnetic flea. The integrity of each membrane was assessed by measurement of electrical impedance prior to application of test substance. Membranes with an impedance of ≥6 k-ohms (rat) and ≥17 k-ohms (human) were considered intact and retained for use on study. Following overnight skin membrane equilibration, the receptor fluid was replaced with 50% (v/v) ethanol in water and the test formulations were applied *via* the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

   The test substance, cyantraniliprole, was applied as the undiluted concentrate at 200 g ai/L and as a 1 g ai/L aqueous dilution. Penetration and absorption were followed using [^{14}C]-cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied at a rate of 10 µL/cm² to two groups of 6 skins per dose level per species. The amount of cyantraniliprole applied per area of skin was approximately 2000 µg/cm² and 10 µg/cm² for the 200 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. At 6 hours, the skin surface of all groups was washed, and one group of 6 skins per dose level per species was terminated to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining 6 skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

II. RESULTS AND DISCUSSION

A. CYANTRANILIPROLE, 200 G CYANTRANILIPROLE/L UNDILUTED CONCENTRATE

The penetration rate for [^{14}C]-cyantraniliprole from the Cyantraniliprole 200 g/L SC undiluted concentrate (200 g cyantraniliprole/L) during the initial 6-hour exposure was only measurable for rat skin (2.93 µg equiv/cm²/h). By the end of the 6-hour exposure period (0 hours post-exposure), the cumulative amount penetrated per area was only quantifiable for rat skin (20.6 µg equiv/cm²) (Table 2). Total penetration, receptor fluid only, represented 1.01% of the applied dose for rat skin; radiolabeled cyantraniliprole from the Cyantraniliprole 200 g/L SC undiluted concentrate did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (Figure 1). Washing of the skin at 6 hours removed >89% of the applied dose exclusive of species, which represented a majority of the unabsorbed dose (Table 3). At the end of the 6-hour exposure period, tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via* the skin, removed 3.73% and 1.09% of the applied dose from rat and human skin, respectively; of the total dose retained in the stratum corneum slightly more was contained in tapes 1 and 2 from human skin (~87%) than from rat skin (~54%). The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 33-fold greater for rat skin (3.94%) than for human skin (0.12%) (Table 3).

Post-exposure, a portion of the dose contained in rat skin continued to partition into the receptor fluid but at a reduced rate compared to the rate of penetration during the 6-hour exposure; radiolabeled cyantraniliprole did not penetrate human skin over the course of the experiment (Table 4). The maximum absorbable dose 18 hours post-exposure was 3.69% and 0.24% for rat and human skin, respectively. (Table 5).

Table 2
Penetration kinetics of [^{14}C]-cyantraniliprole from Cyantraniliprole 200 g/L SC, 200 g Cyantraniliprole/L undiluted concentrate, 0-6 hours (0-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	NA	[^{14}C]-cyantraniliprole of the undiluted concentrate did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase as shown in Figure 1.
1	7.02±2.86	
2	5.82±0.77	
4	12.8±1.59	
6 (end exposure)	20.6±7.43	
Penetration rate, 0.5-6 hours[a] (µg equiv./cm^2/h)	2.93	

[a] Slope of mean data, 0.5-6 hours.
NA = not applicable. Data excerpted from Table 2, page 34 of the report.

Figure 1. (Eexcerpted from page 56 of the study report)

Penetration kinetics of [^{14}C]cyantraniliprole from Cyantraniliprole 200 g/L SC, 200 g cyantraniliprole/L undiluted concentrate, 0-6 hours (0-hour post-exposure group)

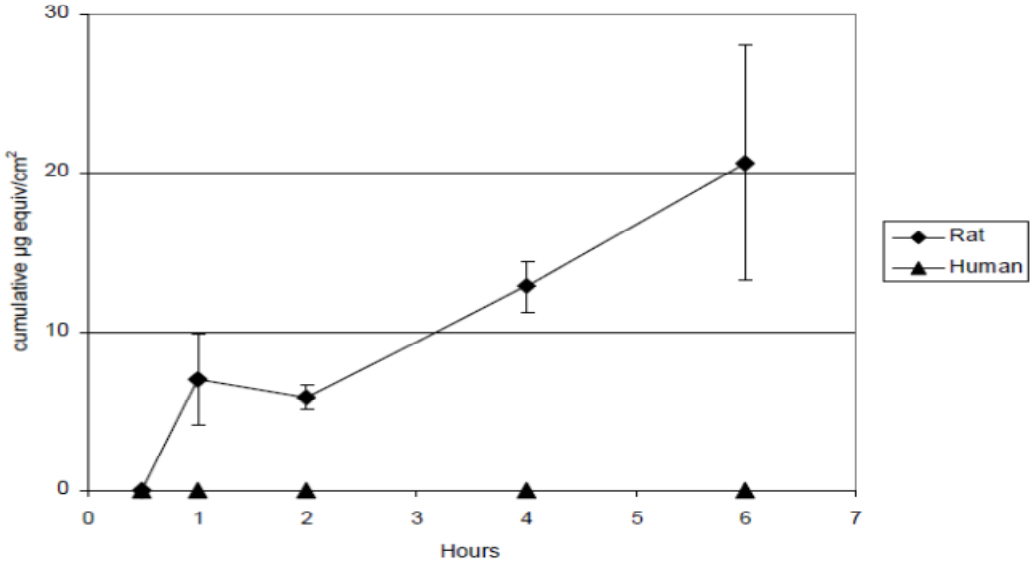


Table 3.
Recovery of total radioactivity at 6 hours following a 6-hour topical exposure to a 200 g Cyantraniliprole/L undiluted concentrate of Cyantraniliprole 200 g/L SC (0-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	1.01±0.36	NA
Total absorbed	1.01±0.36	NA
Absorbable dose		
Receptor fluid	1.01±0.36	NA
Tape-stripped skin	3.10±2.67	0.12±0.11
Total absorbable	3.94±2.34	0.12±0.11
Unabsorbed dose		
Skin wash	89.4±3.03	94.1±3.50
Donor chamber	0.80±0.45	0.53±0.31
Tape strips	3.73±1.56	1.09±0.63
Total unabsorbed	93.9±2.91	96.1±3.71
Total recovered	97.8±1.69	96.2±3.70

NA = not applicable Data excerpted from Table 3, page 35 of the study report.

Table 4
Penetration kinetics of [^{14}C]-cyantraniliprole from Cyantraniliprole 200 g/L SC, 200 g Cyantraniliprole/L undiluted concentrate, 0-24 hours (18-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	NA	Radiolabeled cyantraniliprole in the undiluted concentrate did not penetrate human skin over the course of the experiment.
1	4.03±0.55	
2	7.23±1.42	
4	13.3±2.50	
6 (end exposure)	16.3±6.28	
24 (18 hours post-exposure)	26.5±10.1	
Penetration rate, 0.5-6 hours[a]	2.50	NA
Penetration rate, 6-24 hours[b] (µg equiv./cm^2/h)	0.88	NA

[a] Slope of mean data, 0.5-6 hours [b] Slope of mean data, 6-24 hours

Data excerpted from Table 5, page 37 of the report.

Table 5
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 200 g Cyantraniliprole/L undiluted concentrate of Cyantraniliprole 200 g/L SC (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	Human
Absorbed dose		
Receptor fluid	1.30±0.49	NA
Total absorbed	1.30±0.49	NA
Absorbable dose		
Receptor fluid	1.30±0.49	NA
Tape-stripped skin	2.38±1.83	0.24±0.23
Total absorbable	3.69±1.50	0.24±0.23
Unabsorbed dose		
Skin wash	86.8±5.40	95.4±2.38
Donor chamber	1.06±0.37	1.56±1.48
Tape strips	5.97±4.53	0.84±0.49
Total unabsorbed	93.8±2.56	97.8±1.50
Total recovered	98.4±0.81	98.0±1.49

\ NA= not applicable Data excerpted from Table 6, page 38 of the report.

B. CYANTRANILIPROLE, 1 G CYANTRANILIPROLE/L AQUEOUS DILUTION

The penetration rate for [^{14}C]-cyantraniliprole during the 6-hour exposure period from a 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation was negligible and only measurable for rat skin (0.03 µg equiv/cm²/h) (Table 6 & Figure 2). Cumulative penetration (amount per area) at the end of the exposure was 6.5-fold greater for rat skin (0.20 µg equiv/cm²) than for human skin (0.03 µg equiv/cm²). Total penetration (receptor fluid only) represented 2.86% and 0.49% of the applied dose for rat and human skin, respectively (Table 7). Washing of the skin at the end of the 6 hour exposure removed ≥62% of the applied dose, exclusive of species. Tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via* the skin, removed 22.9% and 22.0% of the applied dose from rat and human skin, respectively; of the total dose retained in the stratum corneum slightly more was contained in tapes 1 and 2 for human skin (~76%) than for rat skin (~56%). The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 2.3-fold greater for rat skin (10.8%) than for human skin (4.70%).

Post-exposure, a portion of the dose contained in rat skin continued to partition into the receptor fluid; radiolabeled cyantraniliprole was not detected in receptor fluid from human skin until the last collection timepoint (18 hours post-exposure) (Table 8). The maximum absorbable dose 18 hours post-exposure was 11.5% and 5.28% for rat and human skin, respectively (Table 6).

Table 6
Penetration kinetics of [^{14}C]-cyantraniliprole from 1 g cyantraniliprole/L aqueous dilution of Cyantraniliprole 200 g/L SC, 0-6 hours (0-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	ND	ND
1	0.04±0.02	
2	0.07±0.04	
4	0.18±0.17	
6 (end exposure)	0.20±0.16	0.03
Penetration rate, 1-6 hours[a] (µg equiv./cm^2/h)	0.03	NA

[a] Slope of mean data, 1-6 hours
Data excerpted from Table 9, page 41 of the study report.
ND = not detected
NA = not applicable

Figure 2.
Penetration kinetics of [^{14}C] cyantranilprole from 1g/L aqueous dilution
0-6 hours exposure group
(Figure excerpted from page 62 of the study report).

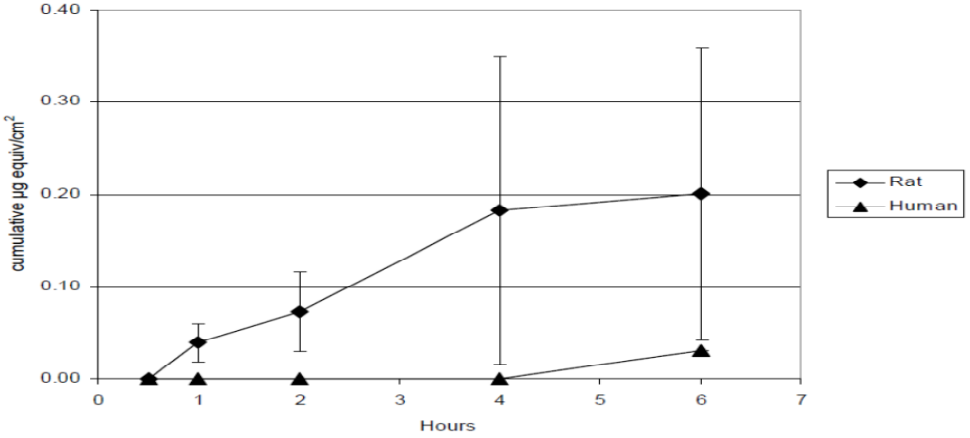


Table 7

Recovery of total radioactivity at 0-hour post-exposure to 1g cyantraniliprole/L aqueous dilution of Cyantraniliprole 200 g/L SC

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	2.86±2.26	0.49±NA
Total absorbed	2.86±2.26	0.49±NA
Absorbable dose		
Receptor fluid	2.86±2.26	0.49±NA
Tape-stripped skin	8.43±11.2	4.62±3.09
Total absorbable	10.8±13.2	4.70±3.02
Unabsorbed dose		
Skin wash	62.0±14.9	69.0±13.4
Donor chamber	1.80±2.05	1.55±1.52
Tape strips	22.9±9.31	22.0±15.8
Total unabsorbed	86.6±20.0	94.3±8.46
Total recovered	**100.8±1.97**	**99.0±6.07**

NA = not applicable Data excerpted from Table 10, page 42 of the study report.

Table 8

Penetration kinetics of [^{14}C]-cyantraniliprole from 1 g cyantraniliprole/L aqueous dilution of Cyantraniliprole 200 g/L SC, 0-24 hours (18-hour post-exposure group)

Time (hour)	**Data expressed in cumulative µg equiv./cm^2**	
	Rat	**Human**
0.5	0.02±NA	ND
1	0.05±0.02	
2	0.11±0.07	
4	0.16±0.12	
6 (end exposure)	0.22±0.15	
24 (18 hours post-exposure)	0.41±0.20	0.05±0.01
Penetration rate, 0.5-6 hours[a]	0.03	NA
Penetration rate, 6-24 hours[b] (µg equiv./cm^2/h)	0.88	NA

[a] Slope of mean data, 0.5-6 hours ND: not detected NA: not applicable

[b] Slope of mean data, 6-24 hours Data excerpted from Table 12, page 44 of the study report.

Table 9
Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 1 g cyantraniliprole/L aqueous dilution of Cyantraniliprole 200 g/L SC (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	5.82±2.80	0.73±0.12
Total absorbed	5.82±2.80	0.73±0.12
Absorbable dose		
Receptor fluid	5.82±2.80	0.73±0.12
Tape-stripped skin	5.71±6.14	4.68±4.32
Total absorbable	11.5±8.00	5.28±4.37
Unabsorbed dose		
Skin wash	61.3±16.0	71.1±16.3
Donor chamber	0.82±0.70	3.55±3.46
Tape strips	25.5±8.86	21.9±9.19
Total unabsorbed	89.3±10.4	96.5±5.54
Total recovered	100.8±3.00	101.8±4.83

Data excerpted from Table 13, page 45 of the study report.

III. Discussion and CONCLUSIONS

The results obtained in this study, using an *in vitro* dermal static diffusion cell model, demonstrate that penetration and absorption of cyantraniliprole from the Cyantraniliprole 200 g/L SC formulation, when applied either as the undiluted concentrate or an aqueous dilution was greatest for rat skin compared to human skin (Table 10). For the cyantraniliprole 200 g/L SC undiluted concentrate, the maximum absorbable dose 18 hours post-exposure (or 24 hours) was 3.69% and 0.24% for rat and human skin, respectively. For 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 200 g/L SC formulation The maximum absorbable dose 18 hours post-exposure (or 24 hours) was 11.5% and 5.28% for rat and human skin, respectively (Table 10).

The human dermal absorption factors were estimated based on the maximum *in vivo* absorption in rats 504 hours following initiation of exposure and the comparative absorption *in vitro* with rat and human skin 24 hours post-exposure (Table 10). Since the 24-hour *in vivo* dermal absorption value for the rat was not available, the 504-hour value (maximum value) was used. The estimated human dermal absorption factors were 0.02% and 0.43% for the concentrate and aqueous dilution, respectively.

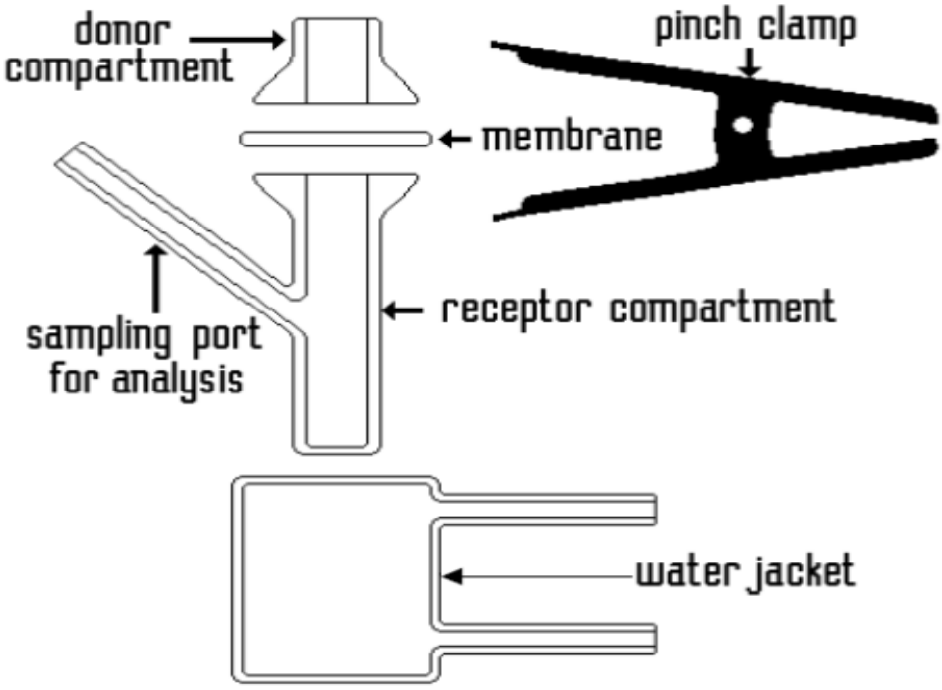
Table 10. Cyantraniliprole 200 g/L SC: Dermal absorption of cyantraniliprole

Study	Sample time	Formulation concentrate	Aqueous solution	Reference
		% Absorbed	% Absorbed	
In vitro - rat skin	6h	3.94	10.8	MRID 48120314 DuPont-24502
	24 h	3.69	11.5	
In vitro - human skin	6 h	0.12	4.7	
	24 h	0.24	5.28	
In vivo - rat	6 h	0.453	0.829	MRID 48120313 DuPont-24501
	504 h	0.342	0.931	
Human dermal absorption factor[a]		**0.02**	**0.43**	

[a] Human absorption factor (% absorbed) ≅ (*in vitro* human % absorption × *in vivo* rat % absorbed)/ *in vitro* rat % absorption; since the % absorbed values for the 6 hr and 504 hr are similar for *in vivo* rat dermal absorption study, the values for 504 hour are used.

Attachment A (Figure excerpted from page 49 of the study report)

Static diffusion cell



Review revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6.2 Comparative dermal absorption, *in vitro* using rat and human skin

Report: Fasano, W.J. (2009); Cyantraniliprole (DPX-HGW86) 100 g/L SE: *In vitro* kinetics of cyantraniliprole in rat and human skin. DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware, USA. Laboratory Report No**.:** DuPont-27073. May 15, 2009. MRID 48120412. Unpublished.

Guidelines: OECD 428 (2004)
SANCO/222/2000 rev.7 (2004)
Deviations: None

GLP: Yes. Signed statements of GLP, Data Confidentiality, and Quality Assurance were submitted in the report.

Executive summary:

In an *in vitro* dermal penetration study (MRID 48120412) using the static diffusion cell model, cyantraniliprole 100 g/L SE, was applied as the suspension emulsion at 100 g cyantraniliprole/L and as a 1 g cyantraniliprole/L aqueous dilution into the diffusion cell. Cyantraniliprole dermal penetration through isolated rat and human skin were followed using [^{14}C]cyantraniliprole, which was uniformly blended into the formulations prior to application. The test articles were applied at a rate of 10 μL/cm^2 to 2 groups of 6 skins per dose level per species (exposure area of 0.64 cm^2 maintained at 32ºC). The amount of cyantraniliprole applied per area of skin was approximately 1000 μg/cm^2 and 10 μg/cm^2 for the 100 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. The receptor fluid was 50% (v/v) ethanol/ deionized water solution. Serial receptor fluid samples (2 × 50 μL) were collected at 0.5, 1, 2, 4, 6 hours (end of exposure) for all replicates, and a final sample was taken at 24 hours for skins held post-exposure. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume. At 6 hours, the skin surfaces of all groups were washed, and one group of 6 skins per dose level per species was terminated (0-hours post-exposure) to determine the distribution of the applied dose at the end of the exposure phase. The remaining six skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

For cyantraniliprole 100 g/L SE undiluted concentrate (100 g cyantraniliprole/L), total penetration, receptor fluid only, represented 6.90% of the applied dose for rat skin (12.2 μg equiv/cm^2/h) by the end of the 6-hour exposure period, but it did not did not penetrate through human skin into the receptor fluid. The cumulative amount penetrated per area was only quantifiable for rat skin (64.4 μg equiv/cm^2). At 6 hours, washing of the skin removed >81% of the applied dose irrespective of species. At the end of the 6-hour exposure period, tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via*

the skin, removed 1.63% and 0.55% of the applied dose from rat and human skin, respectively. The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 10.1-fold greater for rat skin (9.41%) than for human skin (0.93%). Post-exposure, a portion of the dose contained in rat and human skin continued to partition into the receptor fluid but a reduced rate compared to the rate of penetration during the 6-hour exposure. **The maximum absorbable dose 18 hours post-exposure (24 hours) was 13.4% and 2.70% for rat and human skin, respectively.**

For 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation, the total penetration at the end of the exposure was 1.61% of the applied dose for rat skin. Like the concentrate, aqueous dilution did not penetrated through the human skin. The penetration rate for [^{14}C]-cyantraniliprole during the 6-hour exposure period through the rat skin was negligible (0.02 μg equiv/cm^2/h). Washing of the skin at the end of the 6 hour exposure removed ≥71% of the applied dose, exclusive of species. Tape-stripping of the stratum corneum removed 13.1% and 11.8% of the applied dose from rat and human skin, respectively. The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 2.5-fold greater for rat skin (8.40%) than for human skin (3.33%). Post-exposure, a portion of the dose contained in rat and human skin continued to partition into the receptor fluid. **The maximum absorbable dose 18 hours post-exposure (24 hours) was 10.7% and 6.1% for rat and human skin, respectively.**

The results obtained in this study, using an *in vitro* dermal static diffusion cell model, demonstrate that penetration of cyantraniliprole from the Cyantraniliprole 100 g/L SE formulation, when applied either as the undiluted concentrate or an aqueous dilution was greater for rat skin than for human skin. **The *in vitro* dermal absorption data on isolated rat and human skin in combination with the *in vivo* rat dermal absorption data were used to estimate the *in vivo* human dermal absorption factor; the factors were 0.25% for cyantraniliprole 100 g/L SE formulation concentrate and 0.83% for 1g/L aqueous dilution of the concentrate.**

This study is reliable (Acceptable) and fulfilled the OECD guideline (OECD 428 (2004)). *However, the study report should provide the information for the location where the human and rat skin was derived (i.e. abdominal skin).*

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 100 g/L SE
	Lot/Batch #:	HGW86-358
	Purity:	100 g ai./L (undiluted concentrate) 1 g ai./L (field application rate)
	Description:	Liquid
	CAS#:	None for the formulation 736994-63-1 for the active ingredient
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantrnailiprole technical

	Lot/Batch #:	[pyrazole carbonyl-^{14}C]cyantraniliprole abbreviated as [PC-^{14}C]cyantraniliprole, Lot #: 3562-042
	Radiochemical purity:	[PC-^{14}C]cyantraniliprole: radiochemical purity >98%
	Specific activity:	[PC-^{14}C]cyantraniliprole: 44.06 μCi/mg
	Description:	Not specified
	Stability of test compound:	The test material was stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of cyantraniliprole, was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated 100 g/L SE formulation. The blank formulation was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water was used for the field application formulation
5.	Rat skin:	Skin used was from male rats of the Sprague-Dawley strain, Crl:CD®(SD)IGS BR, approximately 6-8 weeks of age. Rats were sacrificed by carbon dioxide asphyxiation and the fur from the dorsal region was carefully shaved using clippers. Any animals showing obvious abrasion within the region of the test skin area were considered unsuitable and discarded. The shaved area was excised, held briefly on wet ice, and then frozen at approximately -20°C until processed.
6.	Human skin:	Samples of human skin from the National Disease Research Interchange, Philadelphia, PA, were stored frozen at approximately 20°C until prepared for use.
7.	Test substance concentrations:	See Table 1.

Table 1
Summary of the formulation, target concentration and skin dose

Formulation	Target concentration	Target skin dose
Cyantraniliprole (concentrate)	100 g ai./L	1000 μg ai./cm^2
Cyantraniliprole (aqueous dilution)	1 g ai./L	10 μg ai./cm^2

B. STUDY DESIGN AND METHODS

1. Study start and completion dates

   February 11,-2009 to March 13,2009

2. Dermal penetration and absorption assay

   The dermal penetration of cyantraniliprole was measured *in vitro* through rat and human skin using a static diffusion cell (Figure 1 of Attachment A). Frozen samples of rat and human skin were thawed and full thickness skin was dermatomed to approximately 450 μm. Each skin membrane was mounted over the receptor chamber of a glass *in vitro* diffusion cell with the stratum corneum uppermost. The receptor chamber was then filled with 0.9% saline and maintained at approximately 32°C for the duration of the experiment. During the exposure phase, the contents of the receptor compartment were continuously stirred using a magnetic flea. The integrity of each membrane was assessed by measurement of electrical impedance prior to application of test substance.

Membranes with an impedance of ≥6 k-ohms (rat) and ≥17 k-ohms (human) were considered intact and retained for use on study. Following overnight skin membrane equilibration, the receptor fluid was replaced with 50% (v/v) ethanol in water and the test formulations were applied *via* the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

The test substance, cyantraniliprole, was applied as the undiluted concentrate at 100 g ai./L and as a 1 g ai./L aqueous dilution. Penetration and absorption were followed using [^{14}C]cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied at a rate of 10 μL/cm^2 to two groups of six skins per dose level per species. The amount of cyantraniliprole applied per area of skin was approximately 2000 μg/cm^2 and 10 μg/cm^2 for the 200 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skins for 6 hours. At 6 hours, the skin surface of all groups was washed, and one group of six skins per dose level per species was terminated to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining six skins at each dose level per species were maintained until 18 hours post-exposure and then terminated. At termination the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

II. RESULTS AND DISCUSSION

A. CYANTRANILIPROLE, 100 G CYANTRANILIPROLE/L UNDILUTED ONCENTRATE

The penetration rate for [^{14}C]cyantraniliprole from the Cyantraniliprole 100 g/L SE undiluted concentrate (100 g cyantraniliprole/L) during the initial 6-hour exposure was only measurable for rat skin (12.2 μg equiv/cm^2/h) (Figure 1 & Table 2). By the end of the 6-hour exposure period (0 hours post-exposure), the cumulative amount penetrated per area was only quantifiable for rat skin (64.4 μg equiv/cm^2). Total penetration, receptor fluid only, represented 6.90% of the applied dose for rat skin; radiolabeled cyantraniliprole from the Cyantraniliprole 100 g/L SE undiluted concentrate did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (Table 3). Washing of the skin at 6 hours removed >81% of the applied dose exclusive of species, which represented a majority of the unabsorbed dose. At the end of the 6-hour exposure period, tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via* the skin, removed 1.63% and 0.55% of the applied dose from rat and human skin, respectively (Table 3). The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 10.1-fold greater for rat skin (9.41%) than for human skin (0.93%).

Post-exposure, a portion of the dose contained in rat and human skin continued to partition into the receptor fluid but at a reduced rate compared to the rate of penetration during the 6-

hour exposure. The maximum absorbable dose 18 hours post-exposure was 13.4% and 2.70% for rat and human skin, respectively (Table 24 & 5).

Table 2
Penetration kinetics of [^{14}C]-cyantraniliprole from Cyantraniliprole 100 g/L SE, undiluted concentrate, 0-6 hours (0-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
	Mean	Mean
0.5	NA	Radiolabeled cyantraniliprole from the Cyantraniliprole 100 g/L SE undiluted concentrate did not penetrate through human skin into the receptor fluid over the 6-hour exposure phase (Figure 1).
1	3.71 ± 1.22	
2	16.7± 5.28	
4	43.4 ± 11.4	
6 (end exposure)	64.4 ± 15.8	
Penetration rate, 0.5-6 hours[a] (µg equiv./cm^2/h)	12.2	

[a] Slope of mean data, 1-6 hours. NA = not applicable

Data excerpted from page 36 of the report

Figure 1.(Excerpted from page 55 of the study report)

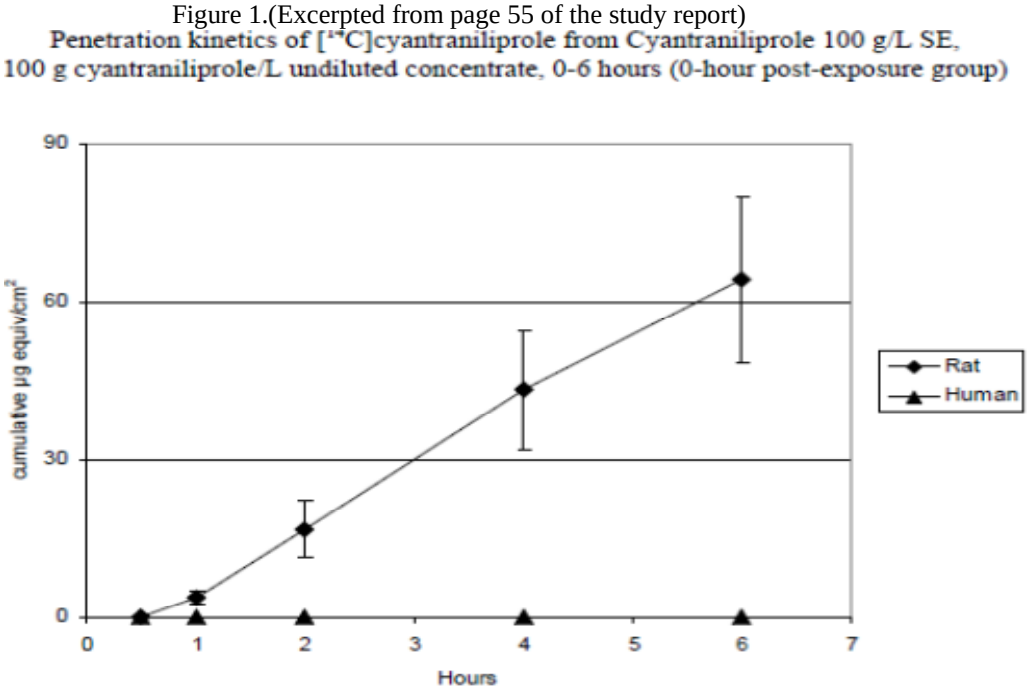


Table 3
Recovery of total radioactivity at 6 hours following a 6-hour topical exposure to a 100 g cyantraniliprole/L undiluted concentrate of Cyantraniliprole 100 g/L SE (0-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	6.90 ± 1.70	NA
Total absorbed	6.90 ± 1.70	NA
Absorbable dose		
Receptor fluid	6.90 ± 1.70	NA
Tape-stripped skin	2.51 ± 0.93	0.93 ± 0.38
Total absorbable	9.41 ± 1.64	0.93 ± 0.38
Unabsorbed dose		
Skin wash	81.2 ± 2.38	93.1 ± 1.73
Donor chamber	0.34 ± 0.22	0.17 ± 0.17
Tape strips	1.63 ± 0.57	0.55 ± 0.26
Total unabsorbed	83.2 ± 2.38	93.8 ± 1.47
Total recovered	92.6 ± 1.93	94.8 ± 1.20

NA = not applicable

Data excerpted from page 36 of the report

Table 4
Penetration kinetics of [^{14}C]-cyantraniliprole from Cyantraniliprole 100 g/L SE, undiluted concentrate, 0-24 hours (18-hour post-exposure group)

Time (hour)	**Data expressed in cumulative μg equiv./cm^2**	
	Rat	**Human**
0.5	NA	NA
1	3.22 ± 0.51	NA
2	13.2 ± 0.65	NA
4	36.6 ± 4.18	NA
6 (end exposure)	53.4 ± 2.33	2.63 ± 0.12
24 (18 hours post-exposure)	84.2 ± 17.5	7.37 ± 7.08
Penetration rate, 1-6 hours[a]	10.2	NA
Penetration rate, 6-24 hours[b] (μg equiv./cm^2/h)	1.71	0.26

[a] Slope of mean data, 0.5-6 hours

[b] Slope of mean data, 6-24 hours

NA = not applicable

Data excerpted from page 38 of the study report.

Table 5. Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 100 g cyantraniliprole/L undiluted concentrate (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	9.02 ± 1.84	0.83 ± 0.80
Total absorbed	9.02 ± 1.84	0.83 ± 0.80
Absorbable dose		
Receptor fluid	9.02 ± 1.84	0.83 ± 0.80
Tape-stripped skin	3.78 ± 0.56	1.87 ± 1.51
Total absorbable	13.4 ± 1.03	2.70 ± 1.65
Unabsorbed dose		
Skin wash	79.4 ± 1.75	91.1 ± 1.14
Donor chamber	0.42 ± 0.19	0.48 ± 0.47
Tape strips	1.22 ± 0.71	0.76 ± 0.37
Total unabsorbed	81.1 ± 1.21	92.1 ± 1.49
Total recovered	94.5 ± 1.28	93.3 ± 2.69

Data excerpted from page 39 of the study report.

B. CYANTRANILIPROLE, 1 G CYANTRANILIPROLE/L AQUEOUS DILUTION

The penetration rate for [^{14}C]-cyantraniliprole during the 6-hour exposure period from a 1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation was negligible and only measurable for rat skin (0.02 µg equiv/cm^2/h) (Figure 2, Table 6). Cumulative penetration (amount per area) at the end of the exposure was only measurable for rat skin (0.14 µg equiv/cm^2) and represented 1.61% of the applied dose (Table7). Washing of the skin at the end of the 6-hour exposure removed ≥71% of the applied dose, exclusive of species. Tape-stripping of the stratum corneum, the primary barrier to systemic uptake of chemicals *via* the skin, removed 13.1% and 11.8% of the applied dose from rat and human skin, respectively. The total absorbable dose at the end of the 6-hour exposure (receptor fluid plus tape-stripped skin) was approximately 2.5-fold greater for rat skin (8.40%) than for human skin (3.33%).

Post-exposure, a portion of the dose contained in rat and human skin continued to partition into the receptor fluid. The maximum absorbable dose 18 hours post-exposure was 10.7% and 6.07% for rat and human skin, respectively (Table 8 & 9).

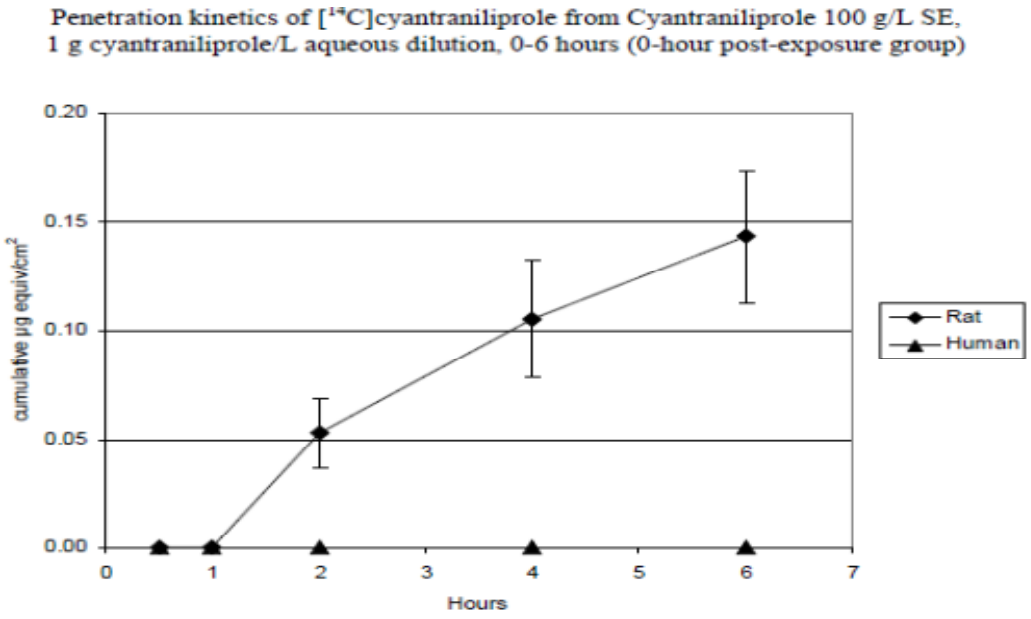
Table 6. Penetration kinetics of [^{14}C]cyantraniliprole from **1 g cyantraniliprole/L aqueous dilution, 0-6 hours (0-hour post-exposure group)**

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	Human
0.5	NA	Penetration was not measurable for human skin at 6 hours of exposure.
1	NA	
2	0.05 ± 0.02	
4	0.11 ± 0.03	
6 (end exposure)	0.14 ± 0.03	
Penetration rate, 2-6 hours[a] (µg equiv./cm^2/h)	0.02	

[a] Slope of mean data, 2-6 hours

NA = not applicable Data excerpted from 43 of the study report.

Figure 2.



(Figure excerpted from page 61 of the study)

Table 7. Recovery of total radioactivity immediately following a 6-hour exposure to a 1 g cyantraniliprole/L aqueous dilution of Cyantraniliprole 100 g/L SE

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	1.61 ± 0.34	NA
Total absorbed	1.61 ± 0.34	NA
Absorbable dose		
Receptor fluid	1.61 ± 0.34	NA
Tape-stripped skin	6.80 ± 3.88	3.33 ± 1.74
Total absorbable	8.40 ± 3.99	3.33 ± 1.74
Unabsorbed dose		
Skin wash	77.0 ± 6.79	71.0 ± 5.80
Donor chamber	0.35 ± 0.50	0.55 ± 0.43
Tape strips	13.1 ± 4.35	11.8 ± 7.97
Total unabsorbed	91.1 ± 8.45	83.9 ± 10.7
Total recovered	99.5 ± 5.76	87.2 ± 10.4

NA = not applicable Data excerpted from page 44 of the study report.

Table 8

Penetration kinetics of [^{14}C]-cyantraniliprole from 1 g cyantraniliprole/L aqueous dilution, 0-24 hours (18-hour post-exposure group)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rat	**Human**
0.5	NA	Not measurable
1	0.04 ± 0.01	
2	0.08 ± 0.03	
4	0.14 ± 0.05	
6 (end exposure)	0.18 ± 0.07	
24 (18 hours post-exposure)	0.38 ± 0.12	0.05 ± 0.01
Penetration rate, 1-6 hours[a]	0.03	NA
Penetration rate, 6-24 hours[b]	0.86	
(µg equiv./cm^2/h)		

[a] Slope of mean data, 0.5-6 hours

[b] Slope of mean data, 6-24 hours

NA = not applicable Data excerpted from page 46 of the study report.

Table 9. Total bsorbable and recovery of radioactivity at 24 hours following a 6-hour topical exposure to a 1 g cyantraniliprole/L aqueous dilution (18-hour post-exposure group)

	Data expressed as a percent of applied dose	
	Rat	**Human**
Absorbed dose		
Receptor fluid	4.26±1.40	0.56±0.12
Total absorbed	4.26±1.40	0.56±0.12
Absorbable dose		
Receptor fluid	4.26±1.40	0.56±0.12
Tape-stripped skin	6.47±2.44	5.79±3.89
Total absorbable	10.7±3.42	6.07±3.98
Unabsorbed dose		
Skin wash	70.4±10.4	67.8±3.86
Donor chamber	0.62±0.58	1.18±0.81
Tape strips	12.0±5.97	6.05±0.91
Total unabsorbed	83.0±10.2	76.1±3.00
Total recovered	93.7±6.88	82.2±4.00

Data excerpted from page 47 of the study report.

III. CONCLUSIONS

The results of this study demonstrated that dermal penetration of cyantraniliprole from the Cyantraniliprole 100 g/L SE formulation when applied either as the undiluted concentrate or an aqueous dilution was greater for rat skin than human skin as summarized in Table 10. An *in vivo* dermal absorption study in rats (MRID 48120413) was also conducted on cyantranilliprole 100 g/L SE formulation. The results are included in Table 10. With the results from the *in vitro* dermal penetration study using isolated rat and human skin and those from *in vivo* rat dermal absorption study, an *in vivo* dermal human absorption factor was estimated for both SE formulation concentrate and aqueous dilution of SE formulation concentrate. Since the 24-hour *in vivo* dermal absorption value for the rat was not available, the 504 hour value (maximum value) was used. The dermal human absorption factors were 0.25% for the concentrate and 0.83% for the aqueous dilution.

Table 10. Summary of dermal absorption of cyantraniliprole 100 g/L SE

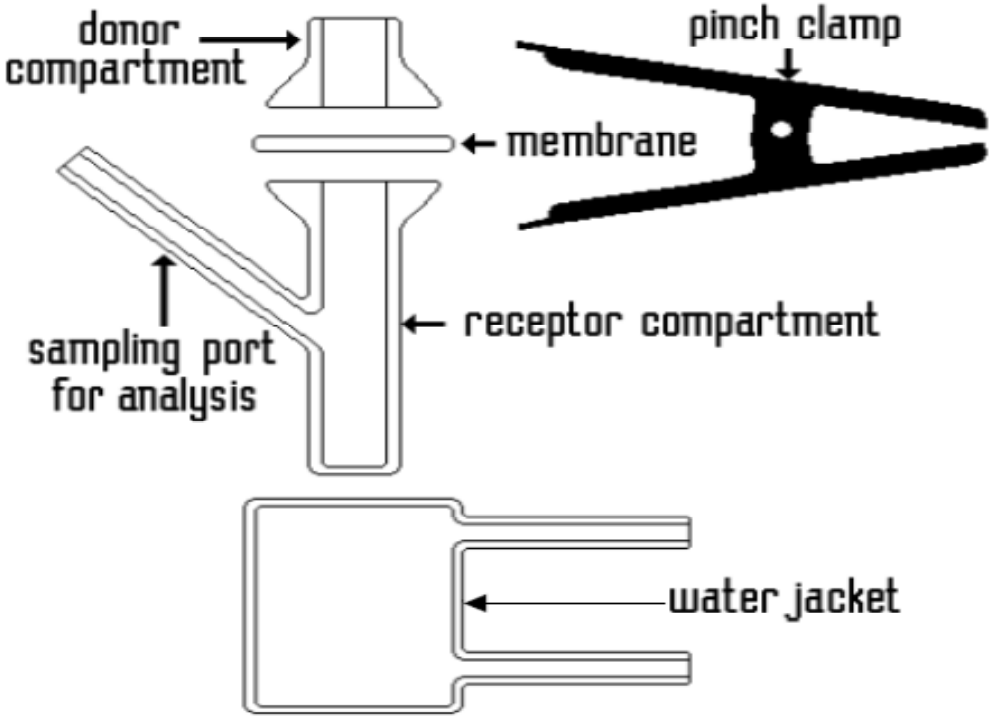
Study	Sample time	Formulation concentrate	Aqueous solution	References
		% Absorbed	**% Absorbed**	
In vitro - rat skin	6h	9.41	8.40	MRID: 48120412 DuPont-27073
	24 h	13.4	10.7	
In vitro - human skin	6 h	0.93	3.33	
	24 h	2.70	6.07	
In vivo - rat	6 h	1.10	0.64	MRID: 48120413 DuPont-27074
	504 h	1.25	1.47	
In vivo Human dermal absorption factor[a]		0.25	0.83	

[a] *In vivo* human dermal absorption factor (% absorbed) = (*in vitro* human % absorption) ×(*in vivo* rat % absorbed/ *in vitro* rat % absorption)

Attachment A

(Figure excerpted from page 51 of the study report)

Figure 1
Static diffusion cell



Revised by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.6 Dermal absorption

IIIA 7.6.1 Dermal absorption, *in vivo* in the rat

Report: Fasano, W.J. (2009); Cyantraniliprole (DPX-HGW86) 100 g/L SE: *In vivo* dermal absorption of cyantraniliprole in the rat. DuPont Haskell Laboratories, Newark, Delaware, USA. Laboratory Report No.: DuPont-27074. May 15, 2009. MRID 48120413. Unpublished.

Guidelines: OECD 427 Section 4 (2004),
OECD 28 (2004)
SANCO/222/2000 rev.7 (2004)

Deviations: None

GLP: Yes. Signed statements of GLP, Data Confidentiality, and Quality Assurance were included in the study report.

Executive summary:

In an *in vivo* dermal absorption study (MRID 48120413), a formulation of cyantraniliprole (100 g/L SE) was applied as the undiluted concentrate at 100 g cyantraniliprole/L and as a 1 g cyantraniliprole/L aqueous dilution to 2 groups of male Crl:CD®(SD)IGS BR rat (8 males/group). Absorption was followed using [^{14}C]cyantraniliprole, which was uniformly blended into the formulations prior to application. The formulated products were applied to a 10.5 cm^2 shaved area on the dorso-lumbar region at a rate of 10 µL/cm^2 to two sub-groups of four rats per dose level. The amount of cyantraniliprole applied per area of skin was approximately 1000 µg/cm^2 and 10 µg/cm^2 for the 100 g/L undiluted concentrate and the 1 g/L aqueous dilution, respectively. The applied formulation remained in contact with the skin for 6 hours. At 6 hours, the application site of all rats was washed with a dilute soap solution, and one group of 4 rats per dose concentration was sacrificed to determine the distribution of the applied dose at the end of the exposure phase (0-hours post-exposure). The remaining 4 rats at each dose level were maintained until 504 hours post-dose (498 hours post-exposure; 21 days). At sacrifice, the application skin site was tape-stripped to remove the stratum corneum and total distribution of the applied material determined for each post-exposure group. The formulation concentrations and application rates were designed to mimic potential field-use exposures.

For the **undiluted concentrate of Cyantraniliprole 100 g/L SE, d**uring the 6-hour dermal exposure only a minor amount of the applied dose was absorbed (0.73%). At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (>86%), while removal of the stratum corneum by tape stripping accounted for approximately 2.17%. Given the amount of dose absorbed (0.73%) and the dose remaining in the tape-stripped skin (0.37%), the total absorbable dose at 6 hours was calculated to be 1.10%. **Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.25% for undiluted concentrate of Cyantraniliprole 100 g/L SE .**

For the **1 g/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation,** only a minor amount of the applied dose was absorbed (0.42%) over the course of a 6-hour dermal exposure. At the end of the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>96%), while removal the stratum corneum by tape stripping accounted for 2.61%. Given the amount absorbed (0.42%) and the dose remaining in the tape-stripped skin (0.23%), the total absorbable dose at 6 hours was calculated to be 0.64%. **Based on a 498-hour recovery/collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.47% 1 g/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation.**

In conclusion, following a 6-hour dermal exposure to Cyantraniliprole 100 g/L SE formulation, when applied either as the undiluted concentrate or as an aqueous dilution, total absorption was low, and maximum absorption, represented as a percent of the applied dose, was higher for the aqueous dilution (1.47%) than for neat undiluted formulation (1.25%). The dermal absorption data from both *in vivo* and *in vitro* dermal absorption studies were used to estimate the *in vivo* dermal absorption factor for human. The estimated factors were 0.25% for cyantraniliprole 100 g/L SE formulation concentrate and 0.83% for aqueous dilution.

This study is reliable (acceptable) and meets the OECD guideline requirements (OECD 427 Section 4 (2004)).

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Cyantraniliprole 100 g/L SE
	Lot/Batch #:	HGW86-358
	Purity:	100 g ai./L (undiluted concentrate) 1 g ai./L (field application rate)
	Description:	White liquid
	CAS#:	None for the formulation 736994-63-1 for the active ingredient
	Stability of test compound:	The test materials were stable over the course of the experiments
2.	Radiolabel test material:	^{14}C-cyantraniliprole
	Lot/Batch #:	[Pyrazole carbonyl-^{14}C]cyantraniliprole abbreviated as PC-^{14}C]cyantraniliprole, Lot#: 3562-042
	Radiochemical purity:	[PC-^{14}C]cyantraniliprole: Radiochemical purity >98%
	Specific activity:	[PC-^{14}C]cyantraniliprole: 44.06 µCi/mg
	Description:	Not specified in report
	Stability of test compound:	The test materials were stable over the course of the experiments
3.	Formulation blank:	The blank formulation, which was devoid of cyantraniliprole, was blended with radiolabeled cyantraniliprole to produce the field application formulation (aqueous dilution). The blank formulation was blended with the non-labeled and radiolabeled ingredients to produce the undiluted concentrated formulation. The blank formulation was stored at room temperature.
4.	Vehicle and/or positive control:	Water for the aqueous dilution
5.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)IGS BR
	Sex	Males
	Age at dosing:	Approximately 6-8 weeks old
	Weight at dosing:	202-213 g
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Acclimation period:	6 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed singly in all-glass metabolism units
4.	Environmental conditions	
	Temperature:	22-24°C
	Humidity:	40-60%

Air changes: Not reported
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

10-February-2009 to 12-March-2009

8 male2. Animal assignment and treatment

On the day prior to dermal dosing, the back and shoulders of each animal was clipped free of hair and the clipped area washed with a 2% Ivory® Soap solution in water. Following shaving and washing, three O-rings (one stacked upon the other) with an internal area of approximately 10.5 cm^2, were glued to the clipped area on the back using Instant Krazy Glue® Gel. The O-rings were then covered with Coban™ body wrap to prevent contamination

Doses applied and target parameters are summarised in Table 1.

Table 1. Study design: Summary of doses applied and target parameters for the *in vivo* assessment of dermal absorption of cyantraniliprole in the rat

Group	Dose concentration of cyantraniliprole	Skin dose level of cyantraniliprole	Number of animals[a]	µCi/rat
A	100 g/L[b]	1000 µg/cm^2	8	8
B	1 g/L[c]	10 µg/cm^2	8	5

[a] Based on four rats at two post-exposure collection timepoints
[b] Rats in group A were exposed to a single application of the undiluted formulation concentrate.
[c] Rats in group B were exposed to a single application of an aqueous dilution of the formulation.

Two sacrifice time intervals for Group A and Group B treatment groups were included in this study:

Interval I: 4 animals were sacrificed 6 hours after dermal application (0 hours post-exposure).

Interval II: 4 animals were sacrificed 504 hours after dermal application (498 hours post-exposure).

3. Dose formulation and analysis

The Cyantraniliprole 100 g/L SE undiluted concentrate was prepared by mixing radiolabeled cyantraniliprole (5.82 mg) with non-radiolabeled cyantraniliprole (355.83 mg) followed by the addition of formulation blank (3.26030 g), and glass beads (5.56310 g @ 0.5-0.75 mm). The ingredients were wet-milled at approximately 3000 rpm for approximately 50 minutes. The blended ingredients were separated from the glass beads by centrifugation and placed into a glass vial.

The Cyantraniliprole 100 g/L SE aqueous dilution was prepared by mixing radiolabeled cyantraniliprole (3.63 mg), formulation blank (0.03250 g), deionised water (3.58800 g), and glass beads (5.64577 g @ 0.5-0.75 mm). The ingredients were wet-milled at approximately 3000 rpm for approximately 50 minutes. The aqueous dilution was separated from the glass beads by centrifugation and placed into a glass vial.

Storage conditions were not specified in the report.

The homogeneity and amount of radiolabeled cyantraniliprole (μCi/g) in each formulated dose was determined by subjecting aliquots of the prepared dose to radioanalysis by liquid scintillation counting (LSC). The concentration of cyantraniliprole in each dose formulation was determined chromatographically by HPLC-UV. The results of homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled cyantraniliprole (μCi/mg) for the formulated doses. The radiochemical purity of the neat radiolabeled cyantraniliprole and the stability of radiolabeled cyantraniliprole in the prepared dose formulations were determined by HPLC-radiochromatography.

4. Dosing

On the day of dosing, the protective gauze wrap was removed and the formulated products were applied within the O-ring area at a rate of 10 μL/cm^2. Following dosing, the dose site was protected with a rigid mesh covering and Coban™ body wrap and each animal was separately housed in an all-glass metabolism cage. The applied formulation remained in contact with the skin for 6 hours. After 6 hours, the skin surface of all rats was washed using natural sponge pieces soaked in 2% Ivory® Soap and water.

5. In-life sample collection

Urine and faeces were collected during the 0-6 hour exposure period, at 6-12 hours, 12-24 hours, and every 24 hours thereafter until sacrifice. The 6-hour aqueous soap solution washes of the application site, the sponges used during the washing, and the body wrap and rigid mesh covering (from the end of the exposure phase and at sacrifice) were collected for analysis.

6. Sacrifice

Animals were exposed to carbon dioxide asphyxiation and exsanguinated *via* cardiac puncture. The application skin site was excised and tape-stripped to remove the stratum corneum. The skin washes, Coban™ body wrap, cage washes, residual feed, skins from the application site, tape strips, skins from a non-dosed area, blood (whole blood, plasma, and red blood cells), and remaining carcass were analysed for total radioactivity.

7. Sample analysis

Urine, skin washes, cage washes, blood plasma were assayed directly by LSC. The protective mesh cover, Coban™ body wrap, O-ring spacers, and tape strips were extracted using acetonitrile and analysed directly by LSC. Faeces, residual feed,

carcasses were homogenised in water and combusted prior to LSC. Skin samples (both the application skin and non-dose skin) and wash sponges pieces were digested in Soluene®-350 and analysed directly by LSC.

8. Statistics

Group data is presented as the mean result.

II. RESULTS AND DISCUSSION

A. RADIOCHEMICAL PURITY, CONCENTRATION, AND STORAGE STABILITY

The purity of radiolabeled cyantraniliprole was >97%. Analyses confirmed that cyantraniliprole was present in the dosing formulations at the appropriate concentrations and with the appropriately amount of radiolabeled test substance. [^{14}C]cyantraniliprole and the formulations were shown to be stable under the conditions of this study.

B. TREATMENT GROUP A – CYANTRANILIPROLE 100 G/L SE UNDILUTED CONCENTRATE

During the 6-hour dermal exposure to the undiluted concentrate of Cyantraniliprole 100 g/L SE, only a minor amount of the applied dose was absorbed (0.73%). At the end of the exposure phase, washing of the skin surface accounted for major portion of the applied dose (>86%), while removal of the stratum corneum by tape stripping accounted for approximately 2.17%. Given the amount of dose absorbed (0.73%) and the dose remaining in the tape-stripped skin (0.37%), the total absorbable dose at 6 hours was calculated to be 1.10%. Based on a 498-hour post exposure recovery/collection period following the 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.25% (Table 2 & Figure 1). It should also be noted that less than 1% of the administered dose was found in the carcass, and this was consistent with rat oral metabolism study results.

Table 2. Absorption of radiolabeled cyantraniliprole by rats exposed to Cyantraniliprole 100 g/L SE undiluted concentrate (Treatment group A)

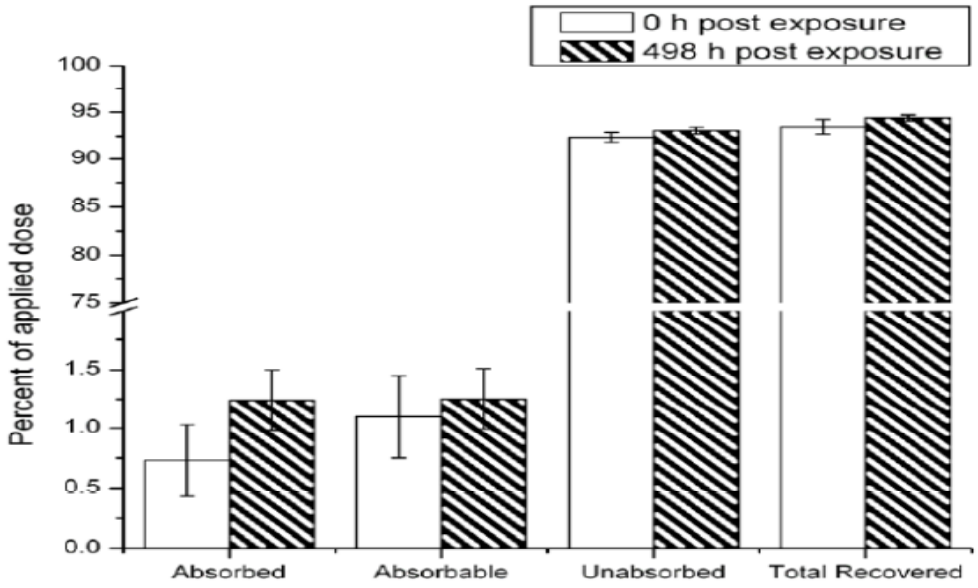
Absorption	Mean percent of administered radioactivity (%)	
	0 h post exposure	498 h post exposure
Unabsorbed dose:		
Skin washes	86.8	89.6
Tape strips (stratum corneum)	2.17	0.13
Protective device[a]	3.45	3.57
Total unabsorbed dose:	92.4	94.4
Absorbed dose:		
Urine	0.05	0.31
Faeces	ND	0.72
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.62	ND
Cagewash	0.05	0.20
Total absorbed dose:	0.73	1.24
Absorbable dose:		
Total absorbed dose	0.73	1.24
Tape stripped skin	0.37	0.01
Total absorbable dose:	1.10	1.25
Total recovered (material balance):	93.5	94.4

[a] Includes O-ring spacers, rigid mesh covering, and Coban™ body wrap

ND: Not detected Data excerpted from Table 3, page 30 of the study report.

Figure 1 (Figure excerpted from page 38 of the study report)

100 g cyantraniliprole/L undiluted concentrate of the Cyantraniliprole 100 g/L SE formulation - absorbed, absorbable, unabsorbed, and total recovered dose, 0 and 498 hours post-exposure



C. TREATMENT GROUP B -CYANTRANILIPROLE 100 G/L SE, 1 G/L AQUEOUS DILUTION

Over the course of a 6-hour dermal exposure to the 1 g/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation, only a minor amount of the applied dose was absorbed (0.42%). At the end of the exposure phase, washing of the skin surface accounted for a vast majority of the applied dose (>96%), while removal the stratum corneum by tape stripping accounted for 2.61%. Given the amount absorbed (0.42%) and the dose remaining in the tape-stripped skin (0.23%), the total absorbable dose at 6 hours was calculated to be 0.64%. Based on a 498-hour recovery/collection period following a 6-hour dermal exposure (~21 days), the maximum absorbable dose was 1.47% (Table 3 & Figure 2).

Table 3.
Absorption of radiolabeled cyantraniliprole by rats exposed to Cyantraniliprole 100 g/L SE, 1 g/L aqueous dilution (Treatment group B)

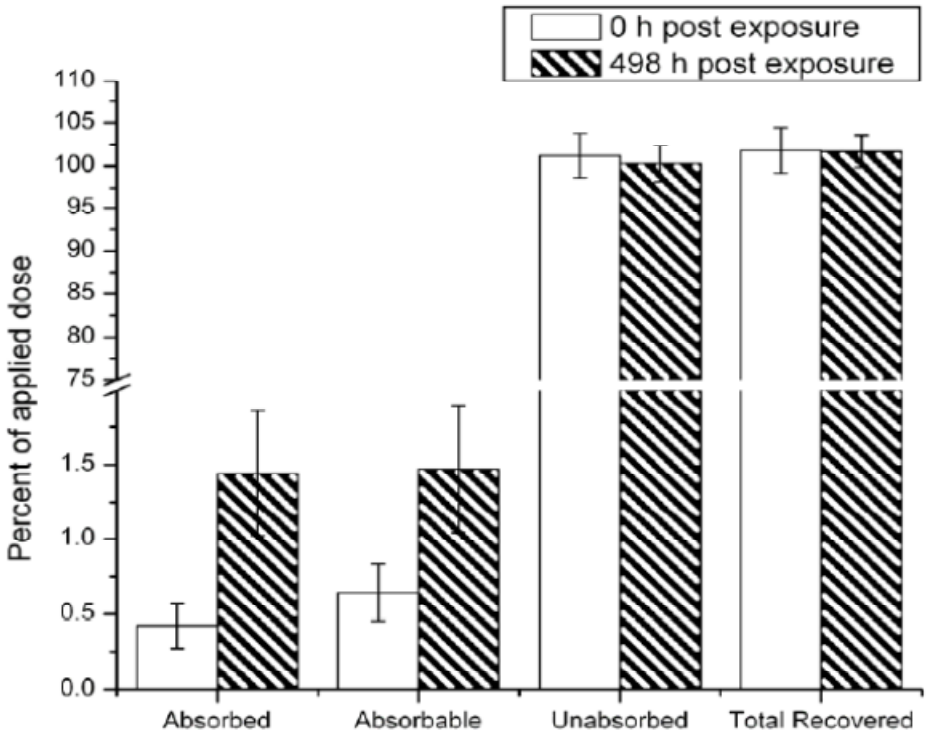
	Mean percent of administered radioactivity (%)	
Absorption:	**0 h post exposure**	**498 h post exposure**
Unabsorbed dose:		
Skin washes	96.4	92.6
Tape strips (stratum corneum)	2.61	1.93
Protective device[a]	2.31	3.89
Total unabsorbed dose:	101.2	100.3
Absorbed dose:		
Urine	0.01	0.36
Faeces	ND	0.98
Whole blood	ND	ND
Other Tissues	ND	ND
Carcass	0.30	ND
Cagewash	0.09	0.14
Total absorbed dose:	0.42	1.44
Absorbable dose:		
Total absorbed dose	0.42	1.44
Tape stripped skin	0.23	0.03
Total absorbable dose:	0.64	1.47
Total recovered (material balance):	101.8	101.7

[a] Includes rigid mesh covering, O-ring spacers and Coban™ body wrap

ND Not detected Data excerpted from Table 5, page 32 of the study report.

Figure 2 (Figure excerpted from page 39 of the study report)

1 g cyantraniliprole/L aqueous dilution of the Cyantraniliprole 100 g/L SE formulation - absorbed, absorbable, unabsorbed, and total recovered dose, 0 and 498 hours post-exposure



III. DISCUSSION AND CONCLUSIONS

In conclusion, following a 6-hour dermal exposure to Cyantraniliprole 100 g/L SE formulation, when applied either as the undiluted concentrate or as an aqueous dilution, total absorption was low, and maximum absorption, represented as a percent of the applied dose, was slightly higher for the aqueous dilution (1.47%) than for neat undiluted formulation (1.25%) Table 4.

Table 4. Summary of *in vivo* dermal absorption for the undiluted concentration and aqueous solution

Dose formulation	Total absorbable dose at end of 6-hour exposure (0-h post exposure)	Maximum total absorbable dose following 498 hour recovery/collection period
Undiluted concentrate	1.10	1.25
Aqueous solution	0.64	1.47

The dermal absorption of cyantraniliprole formulated as Cyantraniliprole 100 g/L SE was evaluated under *in vivo* conditions in the rat and *in vitro* conditions using rat and human skin. Tests were conducted with the undiluted formulation Cyantraniliprole 100 g/L SE concentrate

and with a 1 g/L aqueous dilution. According the report, the formulation concentrations and application rates were designed to mimic potential field-use exposures. In addition, the 1g/L aqueous dilution was the lowest concentration that could be prepared and yet provide a reliable quantitative assessment of absorption based on the specific activity of the radiolabeled cyantraniliprole used. The results of the dermal absorption studies (*in vivo* & *in vitro*) conducted with Cyantraniliprole 100 g/L SE are summarized in Table 5. In addition, *in vivo* human dermal absorption factors were estimated and presented in Table 5.

Table 5. Summary of dermal absorption of cyantraniliprole 100 g/L SE

Study	Sample time	Formulation concentrate	Aqueous solution	Reference
		% Absorbed	% Absorbed	
In vitro - rat skin	6h	9.41	8.40	MRID: 48120412 DuPont-27073
	24 h	13.4	10.7	
In vitro - human skin	6 h	0.93	3.33	
	24 h	2.70	6.07	
In vivo - rat	6 h	1.10	0.64	MRID: 48120413 DuPont-27074
	504 h	1.25	1.47	
In vivo Human dermal absorption factor[a]		0.25	0.83	

[a] I*n vivo* human dermal absorption factor (% absorbed) = (*in vitr*o human % absorption) ×(*in viv*o rat % absorbed/ *in vitro* rat % absorption). Since the 24-hour *in vivo* rat dermal absorption value was not available, the maximum absorption value (504 h) was used in calculating the *in vivo* human dermal absorption factor.

Reviewed by US EPA (Primary Reviewer: Whang Phang, PhD)

IIIA 7.1.1 *In vitro* dermal absorption through human epidermis

Report: Davies D, (2011). CyantraniliproleWG (A16971B) - *In Vitro* Absorption through Human Epidermal Membranes using [^{14}C]-Cyantraniliprole. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Laboratory Report No. JV2133-REG, March 28, 2011. MRID 48432412. Unpublished.

Guidelines: OECD 428 (2004): Human *in vitro* Dermal Absorption Study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In an *in vitro* dermal penetration study (MRID 48432412) using static cell system, cyantraniliprole WG formulation (A16971B: 40% cyantraniliprole) penetration through human epidermal membranes was measured. [^{14}C]-cyantraniliprole was applied as 50% aqueous slurry of the 400 g/kg formulation concentrate (i.e. nominally 200 g cyantraniliprole/L) and as two aqueous dilutions (1/534 and 1/1600 w/w) of the granular formulation concentrate. The test material was applied to epidermal membranes at a rate of 10 µL/cm^2 and the applications were left unoccluded for an exposure period of 24 hours. The human skin membranes were derived from the thigh, back, or abdomen. Each concentration was tested with 6 human skin membranes. Samples of the receptor fluid (50% ethanol in water) were taken at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, & 24 hours after application using an autosampler. The distribution of cyantraniliprole within the test system and a 24 hour absorption profile were determined, using liquid scintillation counting (LSC).

The dose preparations were relatively homogeneous both prior to and during dosing. The radiochemical purity of [^{14}C]-cyantraniliprole in the 1/534 w/w aqueous dilution, was slightly variable prior to and during dosing. The initial purity was 95.5%, which declined to 76.4% over 24 hours. The study author explained that the low purity value seen at 24 hours for the 1/534 aqueous dilution was considered to be an anomaly introduced during analysis.

Formulation concentrate slurry: The absorption rate for the formulation concentrate slurry was fastest during the first 2 hours of exposure.(0.019 µg/cm^2/h); subsequently, it slowed to 0.002 µg/cm^2/h for the remainder of the exposure period (2-24 hours). The amount in the receptor fluid was 0.064 µg/cm^2 over 24 hour exposure period, equivalent to 0.004% of the applied dose. Mean recovery of the applied test material was 103%. Essentially all the applied dose was found in the skin wash 24 hours after application. A mean total of 0.001% of the applied dose was present in the outer layers of the *stratum corneum* (tape strips 1-2). No detectable amount was found in the remaining *stratum corneum*(tap-strips 3-5), and only 0.006% of the applied dose was present in the remaining epidermal tissue. **The total absorbable dose over the entire 24 hour exposure period was 0.010% of the applied dose.**

1/534 w/w aqueous dilution: Mean recovery of the applied test material was 101%. Similar to the formulation concentration slurry, absorption was fastest during the first 2 hours of exposure. Skin washing 24 hours after application removed 99.2% of the applied dose. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 1.36%. A mean total of 0.103% of the applied dose was present in the outer layers of the *stratum corneum* (strips 1 and 2) with 0.041% in the remaining *stratum corneum* (tap-strips 3-5), and only 0.196% of the applied dose was present in the remaining epidermal tissue. **The total absorbable dose over 24 hours was 1.597% of the applied dose.**

1/1600 w/w aqueous dilution: Mean recovery of the applied test material was 97.8%. Absorption of cyantraniliprole in 1/1600 w/w dilution was linear over 24 hours. Skin washing 24 hours after application removed 97.1% of the applied dose. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.274%. The mean percentage of dose present in outer layers of *stratum corneum (*tape strips 1-2) was 0.150% and that in tape strips 3-5 was 0.030%. Only 0.257% of the applied dose was present in the remaining epidermal tissue. **The total absorbable dose over the entire 24 hour exposure period was 0.561% of the applied dose**

The total absorbable dose expressed as percent of the applied dose over 24 hours was 0.010, 1.597 and 0.561% for concentrate slurry, 1/534 w/w dilution, and 1/167 w/w dilution, respectively. Irrespective of the applied dose, greater than 97% of the applied dose remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

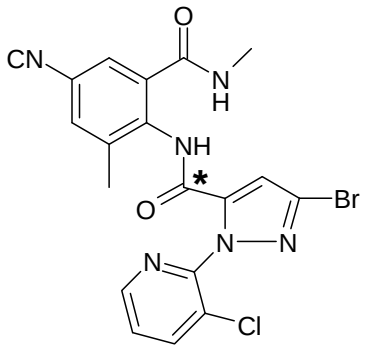
The study is considered reliable and meets the guideline of OECD 428 (2004): Human *in vitro* Dermal Absorption Study

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:	Cyantraniliprole
Other names:	SYN545377, DPX-HGW86-230
Description:	White powder
Lot/Batch number:	HGW86-0603-1
Purity:	93.2% a.i. w/w
Source:	Dupont Crop Protection, Stine-Haskell Research Center, P.O. Box 30, Newark, Delaware 19714-0030, USA.
Stability of test compound:	Expiry date: 24 September 2012
Radiolabelled Test Material:	[Pyrazole carbonyl-^{14}C]-Cyantraniliprole
Synonyms:	[^{14}C]-DPX-HGW86 and [^{14}C]-SYN545377
Specific activity:	50.8 µCi/mg (1.88 MBq/mg)
Radiochemical lot number:	3639107
Chemical Purity:	Not assigned
Radiochemical Purity:	Greater than 95%
Source:	Perkin Elmer Inc., 549 Albany Street, Boston, MA 02118, USA.
Stability of radiochemical:	Expiry date: Not assigned

Structure:



* denotes the position of [^{14}C]-labelled atoms

Representative formulation tested:	Cyantraniliprole: WG formulation A16971B
Blank Formulation:	A16971B Blank (milled) contained all the components of formulation A16901B, except for the active ingredients cyantraniliprole.
Reference number:	DTL reference TS00111/003
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.
Batch number	SMU0DL001
Dose vehicle for spray dilutions:	CIPAC D water
Batch number:	CA1130R
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.

Relevance of Test material to Proposed Formulation: The formulation concentrate and two aqueous dilution doses were prepared in accordance with instructions supplied by the Sponsor, to mimic the commercial formulation and two aqueous spray strength dilutions.

Study Design and Methods:

Study dates: Start: July 26, 2010 End: September 6, 2010

Dose preparation: The three doses were prepared to mimic the commercial A16971B formulation (400 g cyantraniliprole/kg formulation) and two representative aqueous dilutions (1/534 and 1/1600 w/w) using unlabelled cyantraniliprole, [^{14}C]-cyantraniliprole, and formulation blank. As the 400 g cyantraniliprole/kg commercial product is a dry wettable granular formulation, the formulation concentrate dose comprised a 50% aqueous slurry of the dry material, to enable homogeneous doses to be pipetted on the surface of the epidermal membranes. The doses were prepared as close to the time of application as was practicable and were analysed by HPLC to confirm their suitability for use in the study. The radioactivity content and homogeneity of each dose preparation was determined by analysing sub-samples of solvent dilutions by LSC.

Particle size assessment: Each dose was milled during its preparation to ensure that the particle size was similar to the commercial formulation. Particle size measurement was performed using a validated particle sizing procedure, based upon the measurement of particles on microscope slides fitted with a graticule, to ensure that the mean particle size of *circa* 2 µm had been achieved.

Diffusion cell: The type of static glass diffusion cell used in this study has an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL. A diagram of the static glass diffusion cell used in this study is displayed in OECD guideline 428, (2004). Discs of approximately 3.3 cm diameter of prepared skin samples were mounted, dermal side down, in such diffusion cells held together with individually numbered clamps and placed in a water bath

maintained at 32ºC ± 1ºC. Diffusion of cyantraniliprole into and across the epidermal membrane to a receptor fluid was measured during this study.

Receptor fluid: The receptor fluid (50% ethanol in water) was chosen to ensure that the cyantraniliprole would freely partition into this from the skin membrane and never reach a concentration that would limit its diffusion.

Skin preparations: Human skin samples were obtained from a tissue bank. The skin samples were mostly derived from the thigh, back, and the abdomen. Six skin membranes were employed for each test concentration. The samples were immersed in water at 60ºC for 40-45 seconds and the epidermis teased away from the dermis. Each membrane was given an identifying number and stored frozen, at approximately -20ºC, on aluminium foil until required for use.

Skin preparation integrity: Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Rat membranes with a measured resistance of <10 kΩ were regarded as having a lower integrity than normal and not used for exposure to the test materials, due to the possibility of compromised barrier function. Only membranes with an acceptable resistance, thereby showing that they were intact, were used in the study.

Application to the skin: Cells were selected such that each application was represented by six intact membranes from at least two different donors. Each applied dose was weighed and represented 10 µl/cm² (25.4 µl per cell) and was left unoccluded for the exposure period.

Temperature: Throughout the experiment the receptor fluid (50% ethanol in water) was stirred and the epidermal membranes were maintained at a normal skin temperature of 32 ± 1^{o}C in a water bath.

Duration of exposure and sampling: The epidermal membrane was exposed to the test preparations for 24 hours, during which time samples (0.1 mL for formulation concentrate slurry and 0.5 ml for aqueous dilutions) of receptor fluid were taken from the receptor chambers of this static cell system at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application using an autosampler. The receptor fluid in the chambers was stirred continuously and the receptor volume was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume, after each sample had been taken. The samples of receptor fluid were analysed by LSC.

Terminal procedures: The donor chamber was carefully removed and the underside (surface contact with the membrane) wiped with a single sponge pre-wetted with 3% soap solution (3% Teepol L®) in water which was added to the wash sponges (below). The donor chambers were washed with acetonitrile and the sample of the washing analysed for [^{14}C]-cyantraniliprole by LSC.

The epidermal surface of the skin was gently washed by swabbing the application site with natural sponges pre-wetted with a solution of 3% Teepol L® in water. Following assessment of radioactivity levels on the skin surface with a Geiger counter, sponges pre-wetted with water, were used to further swab the surface. The sponges were digested in Soluene 350® and made up

to a recorded volume. A sample of the digest was taken for analysis by LSC. The surface of the skin was allowed to dry naturally.

To assess penetration through human *stratum corneum*, successive layers of the skin surface were removed by the repeated application of adhesive tape (Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips. A strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove the *stratum corneum*. The adhesive strips were soaked individually in acetonitrile to extract any test material. The extracts were sequentially numbered and analysed by LSC.

Analysis: All components of the test system (e.g. receptor fluid, skin wash, donor chamber and epidermis) were analysed by LSC and the recovery determined.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of cyantraniliprole in the receptor solution in terms of µg/cm^2, 'percentage of dose absorbed' and rates of absorption (µg/cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg/cm^2) and 'percentage of applied dose' (see Tables below). Results presented in the report were generated using an Excel spreadsheet developed at DTL.

Definition of total absorbable and unabsorbed test material: The absorbable test material included cyantraniliprole detected in the receptor fluid, in the 3-5 tap-strips of the *stratum corneum,* and in the remaining epidermal membrane. The unabsorbed test material included material removed from the surface of the epidermis by the washing procedure, found in the 1-2 tap-strips of the *stratum corneum,* and that found in the donor fluid

RESULTS AND DISCUSSION

Achieved doses: LSC analysis of the dose preparations confirmed that the dose levels achieved were 214, 0.624 and 0.207 g cyantraniliprole/L for the formulation concentrate slurry and two aqueous dilutions (1/534 w/w and 1/1600 w/w), respectively. The specific activity values for [^{14}C]-cyantraniliprole in the formulation concentrate slurry, 1/534 and 1/1600 w/w aqueous dilutions, expressed as dpm/µg of cyantraniliprole were 1227, 112786 and 112800 dpm/µg, respectively. The dose preparations were considered to be homogeneous and acceptable for use in these experiments as the percentage relative standard deviation (%RSD) for all preparations was less than 3%.

Stability of [^{14}C]-cyantraniliprole in dose preparations: Cyantraniliprole, when formulated as the formulation concentrate slurry and 1/534 w/w aqueous dilution were relatively stable by HPLC. The radiochemical purity of [^{14}C]-cyantraniliprole in the formulation concentrate slurry was 93.2 % immediately following preparation and was 96.3% after 48 hours. For the 1/534 w/w aqueous dilution, purity values of 95.5% and 76.4% were achieved at 0 and 24 hours, respectively. For the 1/1600 w/w aqueous dilution, the radiochemical purity values were 85.9% and 91.5% at 0 and 18 hours, respectively. The study author explained that the low purity value seen at 24 hours for the 1/534 aqueous dilution was considered to be an anomaly introduced during analysis, and provided additional explanation relating to "pre-dilution (and lower radioactivity content)". The additional

explanation was not clear and did not settle the issue in the opinion of the US EPA reviewer. However, this shortcoming did not significantly interfere with the interpretation of the final results.

Particle size: The optimum particle size range, based on the normal manufacture process for this product, was *circa* 2 µm. The mean particle size achieved for the formulation concentrate slurry was 1.70 µm. For the 1/534 and 1/1600 w/w aqueous dilutions, the mean particle sizes achieved were 2.14 and 2.05 µm, respectively. The particle size of the formulated test materials was therefore considered to be acceptable for the purposes of this study.

Absorption from the formulation-concentrate slurry:

Absorption of cyantraniliprole in the formulation concentrate was fastest during the first 2 hours of exposure with a rate of 0.019 µg/cm^2/h; for the remaining exposure period (2-24 hours), it slowed to approximately 0.002 µg/cm^2/h (Table 1). The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.040, 0.025 and 0.059 µg/cm^2, respectively. The amount absorbed over the entire 24 hour exposure period was 0.064 µg/cm^2 (0.004% of the applied dose).

Table 1: Summary of Cyantraniliprole absorption through human epidermis (in receptor fluid)

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates		Mean Dose Absorbed		
	Time period (h)	Absorption rate (µg/cm^2/h)	Time (h)	Amount (µg/cm^2)	Percentage
Formulation concentrate slurry (214 g Cyantraniliprole/L)	0-2	0.019*	6	0.040*	0.002*
n=6	2-24	0.002*	8	0.025*	0.001*
	0-24	0.002*	10	0.059*	0.003*
			24	0.064	0.004*
1/534 w/w dilution (0.624 g Cyantraniliprole/L)	0-2	0.019	6	0.051	0.823
N=6	2-24	0.002	8	0.056	0.902
	0-24	0.003	10	0.060	0.963
			24	0.085	1.36
1/1600 w/w dilution (0.207 g Cyantraniliprole/L)	0-2	0.0002	6	0.002	0.093
n=4	2-24	0.0002	8	0.002	0.110
	0-24	0.0002	10	0.003	0.134
			24	0.006	0.274

Values between the mean LOD and mean LOQ flagged with * have been used as positive values.
Data excerpted from page 29 of the study report.

Absorption from the 1/534 w/w aqueous dilution:

Similar to concentration slurry, absorption of cyantraniliprole in the 1/534 w/w dilution was fastest during the first 2 hours at a rate of 0.19 μg/cm^2/h. Subsequently, the rate dropped to 0.002 μg/cm^2/h (Table1). The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.051, 0.056 and 0.060 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.823, 0.902 and 0.963%. The amount absorbed over the entire 24 hour exposure period was 0.085 μg/cm^2 (1.36% of the applied dose).

Absorption from the 1/1600 w/w aqueous dilution:

Absorption of cyantraniliprole of the 1/1600 w/w aqueous dilution was linear over the entire test period (24 hours) (Table 1). The absorption rate was 0.0002 μg/cm^2/h between 0-24 hours. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.002, 0.002 and 0.003 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.093, 0.110 and 0.134%. The amount absorbed over the entire 24 hour exposure period was 0.006 μg/cm^2 or 0.274% of the applied dose.

MASS BALANCE AND CYANTRANILIPROLE DISTRIBUTION

Formulation-concentrate slurry:

Mean recovery of the applied test material was 103% (Table 2). Essentially all the applied dose was found in the skin wash, 24 hours after application. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.004%. A mean total of 0.001% of the applied dose was present in the outer layers of the *stratum corneum* (tape strips 1-2); no detectable amount was found in the remaining *stratum corneum*(tap-strips 3-5) and only 0.006% of the applied dose present in the remaining epidermal tissue. The total absorbable dose was 0.010% of the applied dose.

Table 2: Summary of cyantraniliprole distribution from the formulation concentrate slurry – 24 hour exposure

Test Compartment (n=6)	Mean μg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	<LOD	<LOD
Skin wash	1861	103
Stratum corneum (tape strips 1-2)	0.026*	0.001*
Stratum corneum (tape strips 3-5)	<LOD	<LOD
Remaining epidermal membrane	0.111	0.006
Receptor fluid	0.064*	0.004*
Total absorbable	**0.175**	**0.010**
Total recovered	1861	103

Values between the mean LOD and mean LOQ have been flagged with * and used as positive values.
Total absorbable = Stratum corneum (tap strips 3-5) + Remaining epidermal membrane + Receptor fluid
Data excerpted from page 30 of the study report.

1/534 w/w aqueous dilution:

Mean recovery of the applied test material was 101% (Table 3). Skin washing 24 hours after application removed 99.2% of the applied dose. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 1.36%. A mean of 0.103% of the applied dose was present in the outer layers of the *stratum corneum* (strips 1 and 2) with 0.041% in the remaining *stratum corneum* (tap-strips 3-5), and only 0.196% of the applied dose was present in the remaining epidermal tissue. The total absorbable dose over 24 hours was 1.597% of the dose.

Table 3: Summary of cyantraniliprole distribution from the 1/534 w/w aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	0.001*	0.023*
Skin wash	6.19	99.2
Stratum corneum (tape strips 1-2)	0.006	0.103
Stratum corneum (tape strips 3-5)	0.003	0.041
Remaining epidermal membrane	0.012	0.196
Receptor fluid	0.085	1.36
Total absorbable	**0.100**	**1.597**
Total recovered	6.30	101

Values between the mean LOD and mean LOQ have been flagged with * and used as positive values.
Total absorbable =Stratum corneum (tap strips 3-5) + Remaining epidermal membrane + Receptor fluid
Data excerpted from page 30 of the study report.

1/1600 w/w aqueous dilution:

Mean recovery of the applied test material was 97.8%. Skin washing 24 hours after application removed 97.1% of the applied dose (Table 4). The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.274%. The mean percentage of dose present in outer layers of *stratum corneum (*tape strips 1-2) was 0.150% and that in tape strips 3-5 was 0.030%. Only 0.257% of the applied dose was present in the remaining epidermal tissue. The total absorbable dose over a 24-hour exposure period was 0.561% of the applied dose.

Table 4: Summary of cyantraniliprole distribution from the 1/1600 w/w aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	<LOD	<LOD
Skin wash	2.01	97.1
Stratum corneum (tape strips 1-2)	0.003	0.150
Stratum corneum (tape strips 3-5)	0.001	0.030
Remaining epidermal membrane	0.005	0.257
Receptor fluid	0.006	0.274
Total absorbable	**0.012**	**0.561**
Total recovered	2.02	97.8

Total absorbable dose=Stratum corneum (tap strips 3-5) + Remaining epidermal membrane + Receptor fluid
Data excerpted from page 30 of the study report.

CONCLUSION: The values for the total absorbable dose expressed as percent of the applied dose over 24 hours for concentrate slurry, 1/534 w/w dilution, and 1/1600 w/w dilution, are summarized in the following table:

	Formulation concentrate slurry	**1/534 w/w aqueous dilution**	**1/1600 w/w aqueous dilution**
Total absorbable dose (% of the applied dose)	**0.010**	**1.597**	**0.561**

Irrespective of the applied dose, greater than 97% of the applied dose remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

The study is considered reliable and meets the guideline of OECD 428 (2004): Human *in vitro* Dermal Absorption Study

Revised review by US EPA (Primary Reviewer: Whang Phang, PhD).

IIIA 7.1 Dermal Absorption

IIIA 7.1.1 *In vivo* dermal absorption in the rat

Report: Runacres S and Harris S (2011). Cyantraniliprole/Thiamethoxam WG (A16901B) - *In vivo* Dermal Absorption in the Rat from WG formulation A16901B. Quotient Bioresearch (Rushden), Pegasus Way, Crown Business Park, Rushden, Northamptonshire, NN10 6ER, UK. Quotient Bioresearch Report No. SGA/56; Syngenta File No. A16901B_1005. March 30,2011. MRID 48432511. Unpublished.

Guidelines

OECD 427 (2004): Skin absorption: *in vivo* method.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In an *in vivo* dermal absorption study on cyantraniliprole granular formulation (20% cyantraniliprole and 20% thiamethoxam), 16 male Han Wistar (Wistar CrL: WI(Han)) rats/dose group were exposed to an aqueous slurry (50:50 w/w) of the granular formulation concentrate (A16901B) and two aqueous dilutions of the granular formulation (nominally 1/20 and 1/267 dilutions) for 6 hours. A volume of 100 µL of the prepared dose was applied to 10 cm^2 of the clipped skin of each animal, which corresponded to nominal doses of 10.0 mg 1.0, and 0.075 mg cyantraniliprole per rat (formulation concentrate slurry, 1/20 and 1/267 dilutions of the granular formulation respectively). After application, each dose site definition device was covered with non-occlusive gauze. The dermal absorption of radioactivity was determined immediately following the 6 hour exposure interval and at subsequent assessment intervals of 24, 72 and 120 hours.

Rats were housed individually in metabolism cages for the collection of excreta. After the 6 hour exposure interval, the application sites on all rats were washed with sponges wetted with soap solution and water to remove the unabsorbed dose. Urine and feces were collected from each cage after the skin wash and at intervals for the duration of each experiment. A terminal cage wash was collected from each cage after the final excreta collection. Four rats per dose group were terminated immediately after the 6 hour exposure interval and after 24, 72 and 120 hours. Cardiac blood samples were collected under terminal anaesthesia. The liver, kidneys, gastro-intestinal tract and representative samples of adipose tissue and muscle were taken for analysis. Each application skin site was excised and sequentially tape-stripped to remove the *stratum corneum*. All samples, including residual carcasses were analysed for radioactivity.

The group mean achieved doses applied were 11.65 mg, 1.00 mg and 0.06 mg cyantraniliprole per rat for the formulation concentrate slurry, 1/20 dilution and 1/267 dilution respectively. These achieved doses were comparable to the nominal doses 10.0, 1.0, and 0.075 mg cyantraniliprole per rat for formulation concentrate slurry, 1/20 dilution, and 1/267 dilutions of the granular formulation, respectively.

The recoveries have been reported as percentages of the applied radioactive dose. To allow direct sample comparison, the recoveries of the applied radioactivity were also normalized to 100% recoveries. Residues of radioactivity which remained in the skin after washing have been reported as potentially absorbable dose.

Following a 6 hour exposure to the **formulation concentrate slurry**, at least 98% of the applied radioactivity was readily washed from the skin surface. Immediately after the 6 hour exposure interval, a mean of 0.1% of the dose remained associated with the application site (potentially absorbable); this declined with time to less than 0.01% after 72 hours. The mean absorbed dose was 0.2% after 6 hours, 0.1% after 24 and 72 hours, and 0.2% after 120 hours.

Following a 6 hour exposure to the **1/20 dilutio**n, at least 96% of the applied radioactivity was readily washed from the skin surface. Immediately after the 6 hour exposure interval, a mean of 1.4% of the dose remained associated with the application site (potentially absorbable); this declined with time and was approximately 0.1% after 120 hours. The mean absorbed dose was 1.6% after 6 hours, 0.9% after 24 hours, and 0.4% after 72 and 120 hours.

Following a 6 hour exposure to the **1/267 dilution**, a mean of at least 94% of the applied radioactivity was readily washed from the skin surface. Immediately after the 6 hour exposure interval, a mean of 1.2% of the dose remained associated with the application site (potentially absorbable); this declined with time to approximately 0.3% after 120 hours. The mean absorbed dose was 0.8% after 6 hours, 1.2% after 24 hours, 1.3% after 72 hours, and 1.6% after 120 hours.

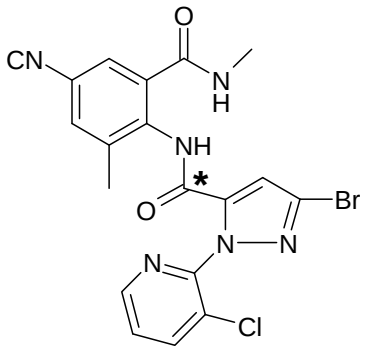
Following a dermal exposure interval of 6 hours to [^{14}C]-cyantraniliprole in either a 50% aqueous slurry of A16901B granular formulation concentrate, or a 1/20 or 1/267 dilution of the granular formulation concentrate, nearly all the applied dose was readily removed from the skin surface by mild skin washing. Irrespective of dose, the extent of absorption over 120 hours expressed as percentage of the applied dose was very low in all animals, accounting for 0.2% for the formulation concentrate slurry, 0.4% for the 1/20 dilution and 1.6% for the 1/267 dilution. At 24 hour exposure, 0.08%, 0.92%, and 1.23% of the applied dose were absorbed with concentrate slurry, 1/20 dilution, and 1/267 dilution, respectively.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:	Cyantraniliprole
Synonyms:	SYN545377 and DPX-HGW86
Description:	White solid
Lot/Batch number:	HGW86-0603-1
Purity:	93.2%
Source:	Du Pont, USA
Stability of test compound:	Expiry date: 24 September 2012
Unlabelled Test Material:	Thiamethoxam (CGA293343 tech.)
Description:	Light beige powder
Lot/Batch number:	SGO7K699E
Purity:	99.0%
Source:	Syngenta
Stability of test compound:	Expiry date: End of March 2011
Radiolabelled Test Material:	[Pyrazole carbonyl-^{14}C]-cyantraniliprole
Synonyms:	[^{14}C]-DPX-HGW86 and [^{14}C]-SYN545377
Description:	White solid
Specific activity:	50.8 µCi/mg (1.88 MBq/mg)
Radiochemical number:	3639107
Chemical Purity:	Not assigned
Radiochemical Purity:	98.1%
Source:	PerkinElmer, USA
Stability of radiochemical:	Expiry date: Not assigned

Structure:



* denotes the position of [^{14}C]-labelled atoms

Representative formulation tested:	Cyantraniliprole:Thiamethoxam (20%:20%) WG formulation A16901B

Blank Formulation:	A16901B blank formulation-milled
Reference number:	DTL reference: TS00111/002/002
Source:	Syngenta
Reanalysis date:	April 2012
Dose vehicle for spray dilutions:	CIPAC-D water
Reference number:	DTL reference: TS00125/001/002
Source:	Syngenta

Relevance of Test material to Proposed Formulation: The formulation concentrate and two aqueous dilution doses were prepared to mimic the commercial formulation and two aqueous end-use dilutions.

The specific activity of [^{14}C]-cyantraniliprole in the three dose preparations were as follows:
formulation concentrate slurry: 71.72 kBq/mg (4303258.8 dpm/mg)
1/20 aqueous dilution of the formulation concentrate: 251.18 kBq/mg (15070872.9 dpm/mg)
1/267 aqueous dilution of the formulation concentrate: 1.88 MBq/mg (112726249.4 dpm/mg)
These specific activity values correspond well with those determined by DTL.

Test Animals:

Species:	Rat
Strain:	Han Wistar (Wistar CrL: WI(Han))
Age/weight at dosing:	Weight range on dosing 216 – 259 g, approximately 6-8 weeks. Group mean weights were 243, 226 and 238 g.
Source:	Charles River UK Ltd, Margate, Kent
Housing:	Individually in metabolism cages
Acclimatisation period:	At least 4 days in stock cages plus one day in metabolism cages
Diet:	Pellet diet (RM1 (E) SQC, Special Diet Services, Witham, Essex, U.K *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 45 - 65% with minor excursions within range of 35-75% Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:
Study dates: Start: 05 August 2010 End: 12 October 2010

Dose rationale: The doses and intervals of exposure were selected to represent typical exposures to the formulation concentrate and end-use aqueous dilutions of 1/20 and 1/267.

Nominal doses: 10, 1.0 and 0.075 mg a.i./10 cm^2 skin.

Achieved doses: 11.7, 1.0 and 0.06 mg a.i./10 cm^2 skin. The achieved doses are the group means of the calculated dose for each rat at each dose level.

Dose volume: 10 µl/cm^2 skin.

Duration of exposure (time from dose to skin wash): 6 hours.

Termination periods (time from dose to sacrifice): 6, 24, 72 and 120 hours. For each dose level, one sub-group of 4 rats was skin washed and terminated immediately following a 6 hour exposure interval. The remaining rats were skin-washed after 6 hours and sub-groups of 4 rats terminated after 24, 72 and 120 hours.

Number of animals/group: 16 male rats per dose level.

Animal Preparation: On the day prior to dosing, the fur behind the shoulders of each rat was clipped and the exposed skin swabbed with acetone to remove sebum. The animals were placed in individual glass metabolism cages. On the day of dosing, a dose site definition device (four silicone rubber O-rings glued together) was glued to the clipped skin. Care was taken to avoid the inclusion of any damaged skin within each defined area. A non-occlusive nylon gauze cover was attached to the top of the dose site definition device after dose application. The total area of skin defined for dose application to each rat was 10 cm^2 per rat.

Dose Preparation, Application and Quantification:
Preparation: The Sponsor provided Dermal Technology Laboratory (DTL) Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK with radiolabelled cyantraniliprole, unlabelled cyantraniliprole and thiamethoxam, a blank formulation of A16901B and CIPAC D water. Three dose preparations, representing the formulation concentrate and the 1/20 and 1/267 aqueous dilutions of the dry granular formulation concentrate, were prepared and supplied by Dermal Technology Laboratory (DTL) Ltd. to mimic the commercial formulation and two aqueous dilutions. As the commercial formulation concentrate is a dry wettable granular (WG) product, a 50:50 w/w aqueous slurry was prepared to facilitate dermal dosing, and this is hereafter referred to as the formulation concentrate slurry. Each dose preparation was divided to enable exactly the same doses to be used synchronously for both the *in vivo* dermal absorption study through rat skin at Quotient and the *in vitro* percutaneous absorption studies through rat and human skin samples done at DTL. The formulation concentrate slurry dose was prepared on 9 August 2010; the 1/20 dilution dose was prepared on 16 August 2010 and the 1/267 dilution dose was prepared on 13 September 2010. Each dose preparation was delivered from DTL to Quotient on day of preparation and was continuously mixed on a magnetic stirrer at *circa* 4°C pending usage. The formulation concentrate slurry and 1/20 dilution formulations were used for administration on days 1 and 2 after receipt, the 1/267 dilution formulation was used on day 1 after receipt.

Application: On the day of dosing (before adhesion of dose site definition devices) each rat was weighed and a record made. Following device adhesion, a positive displacement pipette was loaded with the appropriate formulation (10 µL/cm^2 and 100 µL per rat), any excess formulation was wiped from the pipette tip with tissue and the pipette was weighed. The 100 µL volume was applied to the defined skin area on each rat and the edge of the pipette tip was used to spread the

dispensed dose as evenly as practicable over the defined area, taking care to avoid direct contact of the tip or dose preparation with the application site definition device. The pipette was re-weighed so that the weight of the aliquot applied could be calculated. The pipette tips used during the application were separately retained but not analysed, because the pipette weighing procedure was shown to be accurate. A nylon gauze cover protected each application site for the duration of the exposure interval. The rats were identified by tail numbering (1-16 for the formulation concentrate slurry, 17-32 for the 1/20 dilution and 33-48 for the 1/267 dilution). A record was made of the time of each dose. Immediately after dosing each dosed rat was returned to its metabolism cage.

The dates of dose application were as follows: formulation concentrate slurry, rats 1-4 and 9-12, 10 August 2010; rats 5-8 and 13-16, 11 August 2010: 1/20 dilution, rats 17-20 and 25-28, 17 August 2010; rats 21-24 and 29-32, 18 August 2010: 1/267 dilution, rats 33-48, 14 September 2010.

Quantification: In each experiment the dose preparation was stirred continuously throughout the period of dosing. Samples for [^{14}C]-analysis were taken as dose checks before, during and after the dosing period, to ensure homogeneity and to facilitate dose calculations. For each dose, 100 µL aliquots were transferred from the dose preparation into a volumetric flask and weighed before dilution in acetonitrile for [^{14}C]-analysis. The pipette tips used for the dose checks were separately retained but were not analysed, because the weighing procedure was shown to be accurate. The radiochemical purity of the [^{14}C]-SYN524464 in each dose preparation was determined by TLC and HPLC. The dates of analysis were as follows: formulation concentrate slurry, dose checks 1-5, 10 August 2010; dose checks 6-12, 11 August 2010: 1/20 dilution, dose checks 1-5, 17 August 2010; dose checks 6-12, 18 August 2010: 1/267 dilution, dose checks 1-9, 14 September 2010.

Interim Skin Wash: The application site skin of all rats was washed following the 6 hour exposure interval. The rats terminated at 6 hours were skin washed similarly, with the exception that they were lightly aneasthetized. The remaining rats were sequentially removed from their cages and held, un-anaesthetised, over individual aluminium foil trays to enable the collection of any excreta. The three uppermost O-rings of the application site definition device (with the attached gauze cover) were removed before skin washing (one O-ring remained on the animal). Each application site was washed using nominally 6 pieces of natural sponges pre-wetted with a 3% aqueous soap (Dove liquid) solution and 6 pre-wetted with water, followed by two dry sponges to remove any residual water. Care was taken to avoid the transfer of test substance from the skin surface to the O-ring with the first few sponges and to avoid any loss of the skin washings. All of the sponges used for each rat were retained in a single container for [^{14}C]-analysis. The quality of washing was monitored by the use of a Geiger counter to ensure that as much of the residual radiolabelled dose as practicable was removed from the skin surface. The animals were returned to their appropriate metabolism cages. Any urine and faeces excreted during the washing procedure were added to the respective 0 – 6 hour excreta collections. Urine and faeces were collected from each cage immediately after each skin wash was completed. The foil containers were separately retained but not analysed, because all recoveries of dose were shown to be virtually quantitative.

Terminal Skin Wash: For each dose level, sub-groups of 4 rats were skin-washed and terminated (i) immediately after a 6 hour exposure interval, (ii) 24 hours after dosing, following an interim skin-wash after 6 hours, (iii) 72 hours after dosing, following an interim skin-wash after 6 hours and (iv) 120 hours after dosing, following an interim skin-wash after 6 hours. Each rat was placed on a separate aluminium foil tray and was lightly anaesthetised using Isoflourane. Any residual dose preparation was collected from each application site by washing the skin with liquid soap solution (nominally a 3% aqueous solution of Dove) and water, applied using pieces of pre-wetted natural sponge. Typically 6 sponges of soap solution and 6 with water, followed by two dry sponges to remove any residual water were used to wash each application site, including the inner surface of the remaining O-ring (last few sponges only). Particular care was taken during this procedure to avoid any loss of the skin washings. For each rat, all sponges and washes were collected into a single container. The quality of washing was monitored by the use of a Geiger counter to ensure that as much of the residual radiolabelled dose as practicable was removed from the skin surface. Each remaining dose site definition device ('O' rings) was detached and transferred into the appropriate container used for the nylon gauze cover/upper three rings.

Following skin washing, each rat was kept on the appropriate foil tray and was killed by exsanguination under terminal anaesthesia followed by cervical dislocation. Blood was collected by cardiac puncture and transferred into heparinised tubes. The application site and an annular ring of untreated skin was excised and pinned out on a board. The skin beneath each O-ring, together with a surrounding annular ring of untreated skin, was washed and dried with additional sponges (soap, water and dry), and the sponges stored with the other terminal sponges for each rat. Using successive pieces of adhesive tape, the application skin site was tape-stripped to remove the *stratum corneum* until the epidermis was visible (the first and second tape strips from each animal were transferred into two separate containers, all subsequent tape strips were placed in a third container). The residual skin and the foil from the board were separately retained for each rat. The aluminium foil used for each rat was not analysed, because all recoveries of dose were shown to be virtually quantitative.

Any urine present in the bladder was collected and added to the corresponding excreted sample. The gastrointestinal tract and contents, the liver and kidneys and representative samples of abdominal fat and muscle were removed from each rat and retained. Each residual carcass was retained for analysis. Each sample was transferred to a pre-labelled container for storage prior to analysis.

Excreta Collection: Following dosing, urine and faeces were collected from each cage at 6 hours and 24 hours after dosing and then at daily intervals for the duration of each experiment. At each sample collection time, each cage was rinsed with a small volume of water and the washings added to the corresponding urine container. At the end of each experiment, following the removal of rats and the collection of excreta, metabolism cages were washed with an appropriate volume of ethanol:water (50:50 v/v).

Sample Preparation and Analysis: Urine and faeces were stored at approximately -20°C and cage washes were stored at ambient temperature prior to analysis. Blood samples were refrigerated prior to analysis and tissue samples were stored with residual carcasses at approximately -20°C. Details of sample preparation are provided in Table 1.

Table 1: Sample preparation details for radioactivity analysis

Sample type	Preparation method
Diluted solutions of the dose preparation, urine, cage wash, plasma and other solutions	Direct liquid scintillation counting (LSC)
Faeces	Homogenised to a paste following the addition of a small amount of water. Small samples were analysed by sample oxidation
Dose site definition devices, (O–rings and gauze covers)	Extraction with acetonitrile
Sponges used to wash application sites, *Stratum corneum* on tape strips and skin	Solubilised in Soluene tissue digestant
Whole blood	Sample oxidised
Gastrointestinal tract including contents, abdominal fat, kidney, liver, muscle	Homogenised by scissor mincing for sample oxidation.
Residual carcasses	Solubilised in tissue digestant at 55°C

The radioactivity associated with the dosing formulations, plasma, urine, cage washings, dose site definition device extracts, Soluene digests and carcass digests were determined directly by liquid scintillation counting of known weights of samples. Samples were mixed with Ultima Gold scintillant (Hionic Fluor was used for carcass digests) and counted using a liquid scintillation counter.

Weighed aliquots of whole blood, abdominal fat, kidney, liver, muscle, gastro-intestinal tract and faecal homogenate were combusted in oxygen using a Packard automated sample oxidiser, and the $^{14}CO_2$ produced was trapped with the carbon dioxide absorbent Carbosorb E^+, which was mixed with the scintillant Permafluor E^+ prior to liquid scintillation counting. Combustion efficiency was above 95% for all samples oxidised.

Radioactivity in all samples was quantified directly by liquid scintillation counting (LSC) using a Packard liquid scintillation counter with automatic external standard quench correction. After choosing the optimal channel setting, quench correction curves were prepared from radiochemical standards. The validity of the curves was checked throughout the experiments. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification.

The dates of sample analysis were as follows: formulation concentrate slurry, 13 August 2010 – 17 September 2010; 1/20 dilution, 25 August 2010 – 28 September 2010; 1/267 dilution, 15 September 2010 – 12 October 2010.

RESULTS AND DISCUSSION

Signs and Symptoms of Toxicity: For all dose levels, no adverse clinical effects were observed following dose application.

Summary Tables: Group mean results showing the distribution of radioactivity after 6, 24, 72 and 120 hours are presented as percentages of the applied dose in Tables 2, 3, and 4 for the formulation concentrate, the 1/20 dilution and the 1/267 dilution, respectively.

Table 2: Mean distribution of [^{14}C]-cyantraniliprole residues following the dermal application of A16901B WG formulation concentrate slurry to rat skin

	Recovery (% of applied dose)			
	6 hours (n=3)*	**24 hour (n=4)**	**72 hours (n=4)**	**120 hours (n=4)**
6 hour skin wash	101.96	100.98	97.97	99.15
Terminal skin wash	-	0.33	0.02	<0.01
O-rings	0.31	0.13	0.17	0.10
Stratum corneum (1)	<0.01	<0.01	<LOD	<LOD
***Stratum corneum* (2)**	<LOD	<0.01	<LOD	<LOD
Total unabsorbed	**102.27±3.52**	**101.44±2.13**	**98.16±2.63**	**99.25±1.93**
Stratum corneum (remaining)	0.01	<0.01	<0.01	<0.01
Application site skin	0.08	0.01	<LOD	<LOD
Potentially absorbable	**0.09±0.10**	**0.01±0.01**	**<0.01±0.01**	**<0.01±0.01**
Urine	<0.01	0.01	0.03	0.03
Faeces	<0.01	0.02	0.06	0.16
Cage wash	<LOD	0.04	0.04	0.02
Abdominal fat	<LOD	<0.01	<LOD	<LOD
Kidney	<LOD	<LOD	<LOD	<0.01
Liver	<LOD	<0.01	<LOD	<0.01
Muscle	0.03	<LOD	<LOD	<LOD
G I tract + contents	0.01	0.01	<0.01	<LOD
Carcass + blood	0.20	<LOD	<LOD	<LOD
Absorbed dose	**0.23±0.33**	**0.08±0.07**	**0.13±0.11**	**0.21±0.33**
Total recovered	**102.59±3.32**	**101.52±2.11**	**98.29±2.73**	**99.46±1.84**

* Rat 4M excluded from group means because of an anomalously high recovery of dose
G I Gastro-intestinal
Stratum corneum (1) and (2) = tape strips 1 and 2 Data excerpted from page 32 of the study report.

Table 3: Mean distribution of [^{14}C]-cyantraniliprole residues following the dermal application of a 1/20 dilution of A16901B WG formulation concentrate

	Recovery (% of applied dose)			
	6 hours (n=4)	**24 hour (n=4)**	**72 hours (n=4)**	**120 hours (n=4)**
6 hour skin wash	96.28	98.31	100.36	101.77
Terminal skin wash	-	0.10	0.05	<LOD
O-rings	0.45	1.78	0.14	0.24
Stratum corneum (1)	0.03	<0.01	<0.01	<0.01
Stratum corneum (2)	0.02	<0.01	<LOD	<0.01
Total unabsorbed	**96.78±2.26**	**100.19±0.85**	**100.54±1.34**	**102.01±0.42**
Stratum corneum (remaining)	0.09	0.02	<LOD	0.03
Application site skin	1.27	0.31	0.14	0.07
Potentially absorbable	**1.36±0.60**	**0.32±0.16**	**0.14±0.02**	**0.10±0.02**
Urine	0.03	0.16	0.13	0.15
Faeces	0.01	0.22	0.24	0.23
Cage wash	0.09	0.09	0.01	0.03
Abdominal fat	<0.01	<0.01	<LOD	<0.01
Kidney	<0.01	<0.01	<LOD	<LOD
Liver	0.04	0.04	0.01	<LOD
Muscle	0.02	0.04	0.02	<LOD
G I tract + contents	0.11	0.19	0.03	0.01
Carcass + blood	1.29	0.20	<LOD	<LOD
Absorbed dose	**1.58±0.84**	**0.92±0.56**	**0.44±0.19**	**0.41±0.13**
Total recovered	**99.71±2.36**	**101.44±0.46**	**101.12±1.15**	**102.52±0.39**

G I Gastro-intestinal
Stratum corneum (1) and (2) = tape strips 1 and 2 Data excerpted from page 33 of the study report.

Table 4: Mean distribution of [^{14}C]-cyantraniliprole residues following the application of a 1/267 aqueous dilution of A16901B WG formulation concentrate to rat skin

	Recovery (% of applied dose)			
	6 hours (n=4)	**24 hour (n=4)**	**72 hours (n=4)**	**120 hours (n=3)***
6 hour skin wash	95.71	94.18	95.87	98.84
Terminal skin wash	-	1.34	0.49	0.40
O-rings	0.31	0.42	0.94	0.50
Stratum corneum (1)	0.06	0.05	0.11	0.12
Stratum corneum (2)	0.06	0.08	0.11	0.18
Total unabsorbed	**96.14±1.99**	**96.06±4.78**	**97.51±5.62**	**100.04±2.12**
Stratum corneum (remaining)	0.43	0.38	0.27	0.29
Application site skin	0.76	0.27	0.23	0.01
Potentially absorbable	**1.19±**	**0.65±**	**0.50±**	**0.30±**
Urine	0.12	0.37	0.50	0.45
Faeces	<LOD	0.50	0.61	1.06
Cage wash	0.01	<LOD	0.07	0.02
Abdominal fat	<0.01	<0.01	<0.01	<LOD
Kidney	0.01	<LOD	<LOD	<LOD
Liver	0.10	0.04	<LOD	<LOD
Muscle	0.15	<LOD	<LOD	<LOD
G I tract + contents	0.41	0.25	0.09	0.02
Carcass + blood	0.05	0.08	0.07	0.03
Absorbed dose	**0.83±0.17**	**1.23±0.38**	**1.33±0.68**	**1.58±0.15**
Total recovered	**98.15±2.12**	**97.94±4.69**	**99.33±5.07**	**101.92±2.01**

* Rat 46M excluded from group means as results indicated that some of the dermal dose was ingested
Stratum corneum (1) and (2) = tape strips 1 and 2 Data excerpted from page 34 of the study report.

Definition of Unabsorbed and Absorbed Dose: Radioactivity recovered from the application site skin washings, the dose site definition devices (O-rings and non-occlusive gauze covers) and the first two tape strips of the *stratum corneum* were considered to be unabsorbed. Radioactivity present in the remaining tape strips of the *stratum corneum* and in the underlying application site skin is considered as potentially absorbable, as it is recognized that some of this residue may be absorbed beyond the duration of exposure investigated. The absorbed dose included the radioactivity in urine, faeces, cage wash, residual carcass, g.i. tract (including contents), abdominal fat, kidney, liver and muscle. The carcass values reported as percentages of dose include measurements of radioactivity in the blood samples collected at termination.

Recoveries of applied dose: The group mean percentage recoveries of applied radioactivity at each dose level were 98.2 – 102.6% for formulation concentrate, 99.7 – 102.6% for the 1/20 aqueous dilution and 97.9 – 102% for the 1/267 aqueous dilution. There was good consistency in recoveries within and across groups.

Distribution of radioactivity following application of formulation concentrate slurry: Immediately after the 6 hour exposure interval, group means of 98% or more of the applied dose were readily removed from the skin surface by mild skin washing. A mean of 0.23% of the dose was absorbed during the 6 hour exposure to the formulation concentrate (in animals terminated at 6 hours). Subsequent terminal washes continued to remove further residues from the skin surface, amounting for up to an additional 0.33% of the dose. At each time interval, <0.01% was present in each of the outer layers of the *stratum corneum* (first 2 tape strips), which is considered as part of the unabsorbed dose. The residue in the application site skin and remaining *stratum corneum* was very low over the time course of the study, declining from 0.09% to <0.01% of dose. Over 120 hours after dosing, a group mean of approximately 0.05% of the dose was excreted in urine (including cage wash) and a mean of 0.16% in faeces. The residues in the residual carcass, gastrointestinal tract and contents and excised tissues each represented <0.01% of the dose. Over 120 hours following a 6 hour exposure interval, the total absorbed dose amounted to 0.21% of that applied.

Distribution of radioactivity following application of the 1/20 aqueous dilution: Immediately after the 6 hour exposure interval, group means of 96% or more of the applied dose were readily removed from the skin surface by mild skin washing. Up to a further 0.1% of the dose was removed by the terminal skin washings. Following terminal washing, the [^{14}C]-residues on the O-rings (including non-occlusive gauze covers) accounted for 0.14-1.78% of the dose. The combined skin wash and O-ring measurements of radioactivity amounted to 96.7-102% of the dose. After 6 hours 0.03% and 0.02% of dose were present in the first two strips of the *stratum corneum* and declined to negligible amounts by 24 hours.

In the rats terminated at 6 hours, the radioactivity present in the application site skin and remaining *stratum corneum* amounted to 1.36% of the dose. This declined progressively to 0.32, 0.14 and 0.10% after 24, 72 and 120 hours respectively. At each time less than 0.1% was present in the remaining *stratum corneum* after removal of the first two tape strips. The absorbed dose amounted to 1.58% applied radioactivity after the 6 hour exposure period, 0.92% after 24 hours; 0.44% after 72 hours and 0.41% after 120 hours.

Distribution of radioactivity following application of the 1/267 aqueous dilution: Immediately after the 6 hour exposure interval, group means of more than 94% of the applied dose was readily removed from the skin surface by mild skin washing. An additional 0.4-1.34% was removed by the terminal skin wash. Following the terminal washings, the [^{14}C]-residues on the O-rings (including non-occlusive gauze covers) accounted for 0.31-0.94% of the applied dose. The combined skin washings and O-ring measurements of radioactivity amounted to 95.9-99.8% of the dose. After 6 hours 0.06% of dose was present in each of the first two strips of the *stratum corneum* and this increased to values of 0.12 and 0.18% after 120 hours. In the rats terminated at 6 hours, the radioactivity present in the skin beneath the application sites amounted to 1.2% of the dose, of which 0.43% was present in the remaining *stratum corneum* after the first two tape strips. After 120 hours, 0.3% was present in the application site skin of which 0.29% remained in the *stratum corneum*. The absorbed dose amounted to 0.83% of applied radioactivity immediately after the 6 hour exposure period, 1.23% after 24 hours, 1.33% after 72 hours and 1.58% after 120 hours.

Radioactivity measurements in excreta: For each of the 3 doses, the limited systemic dose was excreted predominantly in faeces with a smaller proportion in urine.

Blood and Plasma: The very low or non-detectable concentrations of radioactivity in blood and plasma are entirely consistent with the low dermal absorption of SYN524464 through rat skin (Table 5).

Radioactivity measurements in tissues: Negligible amounts of dose were present in liver, kidney, muscle and abdominal fat following application of the formulation concentrate slurry or either of the aqueous dilutions, consistent with the very low absorption observed (Table 5).

Table 5: Mean concentrations of [^{14}C]-cyantraniliprole in blood and plasma following the dermal application of a WG formulation (A16901B)

Formulation concentrate slurry	**µg Equivalents of Cyantraniliprole/g**			
	6 hours (n=3)	**24 hours (n=4)**	**72 hours (n=4)**	**120 hours (n=4)**
Whole blood	<LOD	<LOD	<LOD	<LOD
Plasma	<LOD	<LOD	<LOD	<LOD
Kidney	<LOD	<LOD	<LOD	0.015
Liver	<LOD	0.017	<LOD	0.006
Muscle	0.026	<LOD	<LOD	<LOD
Fat	<LOD	0.017	<LOD	<LOD
1/20 Aqueous dilution	**µg Equivalents of Cyantraniliprole/g**			
	6 hours (n=4)	24 hours (n=4)	72 hours (n=4)	120 hours (n=4)
Whole blood	<LOD	0.004	<LOD	<LOD
Plasma	<LOD	0.010	0.005	0.001
Kidney	0.002	0.005	<LOD	<LOD
Liver	0.041	0.046	0.004	<LOD
Muscle	0.001	0.003	0.002	<LOD
Fat	0.016	0.006	<LOD	0.006

1/267 Aqueous dilution	µg Equivalents of Cyantraniliprole/g			
	6 hours (n=4)	24 hours (n=4)	72 hours (n=4)	120 hours (n=3)
Whole blood	0.001	0.003	0.003	0.001
Plasma	0.002	0.005	0.005	0.004
Kidney	0.001	<LOD	<LOD	<LOD
Liver	0.005	0.002	<LOD	<LOD
Muscle	<0.001	<LOD	<LOD	<LOD
Fat	0.003	0.004	0.001	<LOD

CONCLUSION: Following a dermal exposure interval of 6 hours to [^{14}C]-cyantraniliprole in either a 50% aqueous slurry of A16901B granular formulation concentrate, or a 1/20 or 1/267 dilution of the granular formulation concentrate, nearly all the applied dose was readily removed from the skin surface by mild skin washing. Irrespective of dose, the extent of absorption over 120 hours was very low in all animals, accounting for 0.2% of dose for the formulation concentrate slurry, 0.4% for the 1/20 dilution and 1.6% for the 1/267 dilution. At 24 hour exposure, 0.08%, 0.92%, and 1.23% of the applied dose were absorbed with concentrate slurry, 1/20 dilution and 1/267 dilution, respectively.

A human dermal absorption factor was calculated based on the results from the in vitro rat and human skin and in vivo rat dermal absorption studies. The results are summarized in Table 6.

Table 6: Summary of dermal absorption of cyantraniliprole WG Formulation

Study	Sample time	Formulation concentrate % of applied dose absorbed	Aqueous solution (1/267 dilution) % of applied dose absorbed	References
In vivo--rat	24 h	0.08	1.23	MRID: 48432511
In vitro - human skin	24 h	0.003	0.679	MRID: 48432512
In vitro - rat skin	24 h	0.015	13.2	MRID: 48432513
***In vivo* Human dermal absorption factor**[a]		0.016	0.06	

[a] *In vivo* human dermal absorption factor (% absorbed) = (*in vitro* human % absorption) ×(*in vivo* rat % absorbed/ *in vitro* rat % absorption)

Revised review by US EPA (Primary Reviewer: Whang Phang, PhD).

IIIA 7.1.1 *In vitro* dermal absorption through human epidermis

Report: Davies D, (2011a). Cyantraniliprole/Thiamethoxam WG (A16901B) - *In Vitro* Absorption through Human Epidermal Membranes using [^{14}C]-Cyantraniliprole. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Laboratory Report No. JV2139-REG, (Syngenta File No. A16901B_10038) March 28, 2011. MRID 48432512. Unpublished.

Guidelines: OECD 428 (2004): Human *in vitro* Dermal Absorption Study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In an *in vitro* dermal penetration study (MRID 48432512) using static cell system, cyantraniliprole WG formulation (A16901B: 20% cyantraniliprole and 20% thiamethoxam) penetration through human epidermal membranes was measured. [^{14}C]-Radiolabelled cyantraniliprole doses were applied as 50% aqueous slurry of the 200 g/kg formulation concentrate (i.e. nominally 100 g cyantraniliprole/L) and as two aqueous dilutions (1/20 and 1/267 w/w) of the granular formulation concentrate. The doses were applied to epidermal membranes at a rate of 10 µL/cm^2 and the applications were left unoccluded for an exposure period of 24 hours. The skin membranes were mostly derived from the thigh or abdomen. Each concentration was tested with 6 human skin membranes. Samples of the receptor fluid (50% ethanol in water) were taken at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, & 24 hours after application using an autosampler. The distribution of cyantraniliprole within the test system and a 24 hour absorption profile were determined, using liquid scintillation counting (LSC).

The dose preparations were homogeneous both prior to and during dosing. The radiochemical purity of [^{14}C]-cyantraniliprole in the formulation concentrate slurry and the 1/20 w/w aqueous dilution, was greater than 95% both prior to and during dosing. For the 1/267 w/w aqueous dilution, the initial radiochemical purity was 85.8%, which declined slightly to 85.3% over 24 hours.

Formulation concentrate slurry: The mean recovery of the applied test material was 105%, with the majority of the applied dose (104%) found in the skin wash, 24 hours after application. The rate of absorption was fastest during the first hour of exposure (0.018 µg/cm^2/h), and declined to 0.001 µg/cm^2/h for the remainder of the exposure period. The overall absorption rate (0-24 hours) was 0.001 µg/cm^2/h. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.02 µg/cm^2 equivalent to 0.002% of the applied dose at each time point. A mean total of 0.015% of the applied dose was present in the outer layers of the *stratum corneum* (i.e. 0.008% in tape strip 1 and 0.007% in tape strip 2) with 0.001% in the remaining *stratum corneum* and

only 0.004% of the applied dose present in the remaining epidermal tissue. **The amount absorbed over the entire 24 hour exposure period was 0.030 μg/cm^2 equivalent to 0.003% of the applied dose.**

1/20 w/w aqueous dilution: The mean recovery of the applied test material was 99.2% with 98.9% of the applied dose removed by skin washing 24 hours after application. The absorption rate (0.001 μg/cm^2/h) was consistently low over the entire 24 hour exposure period. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.010, 0.012 and 0.015 μg/cm^2, respectively, equivalent to 0.010, 0.012 and 0.015% of the applied dose. A mean total of 0.038% of the applied dose was present in the outer layers of the *stratum corneum* (i.e. 0.026% in tape strip 1 and 0.011% in tape strip 2) with 0.012% in the remaining *stratum corneum* and only 0.088% of the applied dose present in the remaining epidermal tissue. **The amount absorbed over the entire 24 hour exposure period was 0.028 μg/cm^2 (0.028% of the applied dose)**.

1/267 w/w aqueous dilution: The mean recovery of the applied test material was 109% with the majority of the applied dose (108%) found in the skin wash, 24 hours after application. The rate of absorption in the first 2 hours of exposure was 0.008 μg/cm^2/h which declined to 0.001 μg/cm^2/h for the remainder of the exposure period. The overall absorption rate was 0.001 μg/cm^2/h. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.025, 0.027 and 0.029 μg/cm^2, respectively equivalent to 0.437, 0.477 and 0.505% of the applied dose. . The mean percentages of dose present in *stratum corneum* tape strips 1 and 2 were 0.013% and 0.066% respectively, with 0.005% in the remaining *stratum corneum* and only 0.218% of the applied dose present in the remaining epidermal tissue. **The amount absorbed over the entire 24 hour exposure period was 0.040 μg/cm^2 (0.697% of the applied dose).**

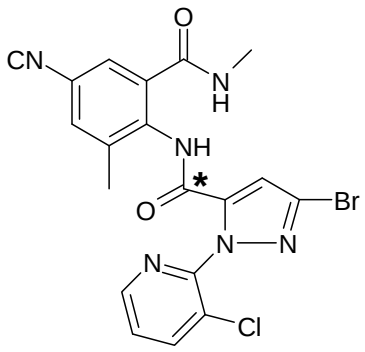
The percentage of each dose absorbed over 24 hours was 0.003, 0.028 and 0.697% for concentration slurry, 1/20 dilution, and 1/267 dilution, respectively. Irrespective of the applied dose, greater than 99% remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:	Cyantraniliprole
Other names:	SYN545377, DPX-HGW86-230
Description:	White powder
Lot/Batch number:	HGW86-0603-1
Purity:	93.2% a.i. w/w
Source:	Dupont Crop Protection, Stine-Haskell Research Center, P.O. Box 30, Newark, Delaware 19714-0030, USA.
Stability of test compound:	Expiry date: 24 September 2012
Unlabelled Test Material:	Thiamethoxam (CGA293343 tech.)
Description:	Light beige powder
Lot/Batch number:	SGO7K699E
Analytical reference number:	10351215
Purity:	99.0% a.i. w/w
Source:	Syngenta Crop Protection Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.
Radiolabelled Test Material:	[Pyrazole carbonyl-^{14}C]-Cyantraniliprole
Synonyms:	[^{14}C]-DPX-HGW86 and [^{14}C]-SYN545377
Specific activity:	50.8 µCi/mg (1.88 MBq/mg)
Radiochemical lot number:	3639107
Chemical Purity:	Not assigned
Radiochemical Purity:	Greater than 95%
Source:	Perkin Elmer Inc., 549 Albany Street, Boston, MA 02118, USA.
Stability of radiochemical:	Expiry date: Not assigned

Structure:



* denotes the position of [^{14}C]-labelled atoms

Representative formulation tested:	Cyantraniliprole:Thiamethoxam (20%:20%) WG formulation A16901B
Blank Formulation:	A16901B Blank (milled) contained all the components of formulation A16901B, except for the active ingredients cyantraniliprole and thiamethoxam.
Reference number:	DTL reference TS00111/002/002
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.
Dose vehicle for spray dilutions:	CIPAC D water
Batch number:	CA1130R
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.

Relevance of Test material to Proposed Formulation: The formulation concentrate and two aqueous dilution doses were prepared in accordance with instructions supplied by the Sponsor, to mimic the commercial formulation and two aqueous spray strength dilutions.

Study Design and Methods:

Study dates: Start: August 8, 2010 End: September 27, 2010

Dose preparation: The three doses were prepared to mimic the commercial A16901B formulation and two representative aqueous end-use dilutions (1/20 and 1/267 w/w) using unlabelled cyantraniliprole, [^{14}C]-cyantraniliprole, unlabelled thiamethoxam and formulation blank. As the 200 g cyantraniliprole/kg commercial product is a dry wettable granular formulation, the formulation concentrate dose comprised a 50% aqueous slurry of the dry material, to enable homogeneous doses to be pipetted on the surface of the epidermal membranes. The doses were prepared as close to the time of application as was practicable and were analysed by HPLC to confirm their suitability for use in the study. The radioactivity content and homogeneity of each dose preparation was determined by analysing sub-samples of solvent dilutions by LSC. The mean concentrations of cyantraniliprole in the formulation

concentrate slurry, 1/20 and 1/267 dilutions were 2.80 mg, 0.256 mg and 0.015 mg/25.4 µL respectively. The formulation concentrate dose was prepared on 8-9 August 2010, with analyses on 9-10 August. The 1/20 dilution dose was prepared on 16 August 2010, with analyses on 16-17 August. The 1/267 dilution dose was prepared and analysed on 13 September 2010.

Supply of dose preparations for synchronised *in vivo* dermal absorption studies:

Immediately after preparation and radiochemical analysis, each of the three doses was divided into two portions, one of which was used in this study. The second portion of each dose was sent, on the afternoon of dose preparation, to Quotient Bioresearch Laboratories for a synchronised *in vivo* dermal absorption study in the rat.

Particle size assessment: Each dose was milled during its preparation to ensure that the particle size was similar to the commercial formulation. Particle size measurement was performed using a validated particle sizing procedure, based upon the measurement of particles on microscope slides fitted with a graticule, to ensure that the mean particle size of *circa* 4-6 µm had been achieved. The formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions were particle sized on 9 August 2010, 16 August 2010 and 13 September 2010, respectively.

Diffusion cell: The type of static glass diffusion cell used in this study has an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL. A diagram of the static glass diffusion cell used in this study is displayed in OECD guideline 428, (2004). Discs of approximately 3.3 cm diameter of prepared skin samples were mounted, dermal side down, in such diffusion cells held together with individually numbered clamps and placed in a water bath maintained at 32ºC ± 1ºC. Diffusion of cyantraniliprole into and across the epidermal membrane to a receptor fluid was measured during this study.

Receptor fluid: The receptor fluid (50% ethanol in water) was chosen to ensure that the cyantraniliprole would freely partition into this from the skin membrane and never reach a concentration that would limit its diffusion.

Skin preparations: Human skin samples were obtained from a tissue bank. The skin samples were mostly derived from the thigh and the abdomen. Six skin membranes were employed for each test concentration. The samples were immersed in water at 60ºC for 40-45 seconds and the epidermis teased away from the dermis. Each membrane was given an identifying number and stored frozen, at approximately -20ºC, on aluminium foil until required for use.

Skin preparation integrity: Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Rat membranes with a measured resistance of <10 kΩ were regarded as having a lower integrity than normal and not used for exposure to the test materials, due to the possibility of compromised barrier function. Only membranes with an acceptable resistance, thereby showing that they were intact, were used in the study.

Application to the skin: Cells were selected such that each application was represented by six intact membranes from at least two different donors. Each applied dose was weighed and represented 10 µl/cm^2 (25.4 µl per cell) and was left unoccluded for the exposure period.

Temperature: Throughout the experiment the receptor fluid (50% ethanol in water) was stirred and the epidermal membranes were maintained at a normal skin temperature of 32 ± 1°C in a water bath.

Duration of exposure and sampling: The epidermal membrane was exposed to the test preparations for 24 hours, during which time samples (0.5 mL) of receptor fluid were taken from the receptor chambers of this static cell system at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application using an autosampler. The receptor fluid in the chambers was stirred continuously and the receptor volume was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume, after each sample had been taken. The samples of receptor fluid were analysed by LSC.

Terminal procedures: The donor chamber was carefully removed and the underside (surface contact with the membrane) wiped with a single sponge pre-wetted with 3% soap solution (3% Teepol L®) in water which was added to the wash sponges (below). The donor chambers were washed with acetonitrile and the sample of the washing analysed for [^{14}C]-cyantraniliprole by LSC.

The epidermal surface of the skin was gently washed by swabbing the application site with natural sponges pre-wetted with a solution of 3% Teepol L® in water. Following assessment of radioactivity levels on the skin surface with a Geiger counter, sponges pre-wetted with water, were used to further swab the surface. The sponges were digested in Soluene 350® and made up to a recorded volume. A sample of the digest was taken for analysis by LSC. The surface of the skin was allowed to dry naturally.

To assess penetration through human *stratum corneum*, successive layers of the skin surface were removed by the repeated application of adhesive tape (Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips. A strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove the *stratum corneum*. The adhesive strips were soaked individually in acetonitrile to extract any test material. The extracts were sequentially numbered and analysed by LSC.

Analysis: All components of the test system (e.g. receptor fluid, skin wash, donor chamber and epidermis) were analysed by LSC and the recovery determined. Samples were analysed between the following dates. Formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions, 12-13 August 2010, 18-23 August 2010 and 15-21 September 2010, respectively.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of cyantraniliprole in the receptor solution in terms of µg/cm^2, 'percentage of dose absorbed' and rates of absorption (µg/cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg/cm^2) and 'percentage of applied dose' (see Tables below). Results presented in the report were generated using an Excel spreadsheet developed at DTL.

Definition of absorbed and unabsorbed test material: The absorbed (systemically available) dose is considered to be the cyantraniliprole detected in the receptor fluid. Material removed from the surface of the epidermis by the washing procedure is regarded as unabsorbed.

Cyantraniliprole recovered from the epidermis at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study.

RESULTS AND DISCUSSION

Achieved doses: LSC analysis of the dose preparations confirmed that the dose levels achieved were 110, 10.1 and 0.577 g cyantraniliprole/L for the formulation concentrate slurry and two aqueous dilutions (1/20 w/w and 1/267 w/w), respectively. The specific activity values for [^{14}C]-cyantraniliprole in the formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions, expressed as dpm/µg of cyantraniliprole were 4289, 15071 and 112800 dpm/µg, respectively. The dose preparations were considered to be homogeneous and acceptable for use in these experiments as the percentage relative standard deviation (%RSD) for all preparations was <5%.

Stability of [^{14}C]-cyantraniliprole in dose preparations: Cyantraniliprole, when formulated as the formulation concentrate slurry and a 1/20 w/w aqueous dilution, was shown to be stable by HPLC for a period of time longer than that used in the study. Radiochemical purities of greater than 95% were seen in all these dose preparations both prior to application and 48 hours post dose preparation. The [^{14}C]-cyantraniliprole formulated as the 1/267 w/w aqueous dilution gave radiochemical purity values of 85.8 and 85.3%, prior to and 24 hours post dose preparation, respectively.

Particle size: The mean particle size range, based on the normal manufacture process for this product, was *circa* 4-6 µm. The mean particle size achieved for the formulation concentrate slurry was 5.03 µm. For the 1/20 and 1/267 w/w aqueous dilutions, the mean particle sizes achieved were 6.26 and 5.38 µm, respectively. The particle size of the formulated test materials was therefore considered to be acceptable for the purposes of this study.

Absorption from the formulation concentrate slurry:

Absorption of cyantraniliprole was fastest during the first hour of exposure. Absorption between 0-1 hour was 0.018 µg/cm^2/h after which it slowed to 0.001 µg/cm^2/h for the remainder of the exposure period (1-24 hours) (Table 1). The absorption rate between 0-24 hours was 0.001 µg/cm^2/h. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.021, 0.022 and 0.023 µg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose all equated to 0.002%. The amount absorbed over the entire 24 hour exposure period was 0.030 µg/cm^2 (0.003% of the applied dose).

Absorption from the 1/20 w/w aqueous dilution:

Because very low amounts of test material were absorbed, absorption rates of cyantraniliprole were very similar over the entire 24 hour exposure period (Table1). The absorption rate between 0-24 hours was 0.001 µg/cm^2/h. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.010, 0.012 and 0.015 µg/cm^2, respectively. These respective amounts expressed as

percentages of the applied dose were 0.010, 0.012 and 0.015%. The amount absorbed over the entire 24 hour exposure period was 0.028 μg/cm^2 (0.028% of the applied dose).

Table 1: Summary of Cyantraniliprole absorption through human epidermis

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates (**n = 6**)		Mean Dose Absorbed (**n = 6**)		
	Time period (h)	Absorption rate (μg/cm^2/h)	Time (h)	Amount (μg/cm^2)	Percentage
Formulation concentrate slurry (101 g Cyantraniliprole/L)	0-1	0.018	6	0.021*	0.002
	1-24	0.0006*	8	0.022	0.002
	0-24	0.0008	10	0.023	0.002
			24	0.030	0.003
1/20 w/w Dilution (9.98 g Cyantraniliprole/L)	0-1	0.001	6	0.010	0.010
	1-24	0.001	8	0.012	0.012
	0-24	0.001	10	0.015	0.015
			24	0.028	0.028
1/267 w/w Dilution (0.577 g Cyantraniliprole/L)	0-2	0.008	6	0.025	0.437
	2-24	0.001	8	0.027	0.477
	0-24	0.001	10	0.029	0.505
			24	0.040	0.697

Values between the mean LOD and mean LOQ flagged with * have been used as positive values.
Data excerpted from page 30 of the study report.

Absorption from the 1/267 w/w aqueous dilution:

Absorption of cyantraniliprole was fastest during the first two hours of exposure. Absorption between 0-2 hours was 0.008 μg/cm^2/h after which it slowed to 0.001 μg/cm^2/h for the remainder of the exposure period (2-24 hours) (Table 1). The absorption rate between 0-24 hours was 0.001 μg/cm^2/h. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.025, 0.027 and 0.029 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.437, 0.477 and 0.505%. The amount absorbed over the entire 24 hour exposure period was 0.040 μg/cm^2 (0.697% of the applied dose).

MASS BALANCE AND CYANTRANILIPROLE DISTRIBUTION

Formulation concentrate slurry:

Mean recovery of the applied test material was 105% (Table 2). The majority of the applied dose, 104% was found in the skin wash, 24 hours after application. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.003%. This percentage equated to 0.030 μg/cm^2. A mean total of 0.015% of the applied dose was present in the outer layers of the *stratum corneum* (i.e. 0.008% in tape strip 1 and 0.007% in tape strip 2)

with 0.001% in the remaining *stratum corneum* and only 0.004% of the applied dose present in the remaining epidermal tissue.

Table 2: Summary of cyantraniliprole distribution from the formulation concentrate slurry – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	2.12	0.210
Skin wash	1054	104
Stratum corneum (tape strips 1-2)	0.151	0.015
Stratum corneum (tape strips 3-5)	0.014*	0.001*
Remaining epidermal membrane	0.041	0.004
Absorbed	0.030	0.003
Total recovered	1056	105

Values between the mean LOD and mean LOQ have been flagged with * and used as positive values.
Data excerpted from page 31 of the study report.

1/20 w/w aqueous dilution:

Mean recovery of the applied test material was 99.2% (Table 3). Skin washing 24 hours after application removed 98.9% of the applied dose. The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.028%. In terms of actual amounts this percentage equated to 0.028 µg/cm^2. A mean total of 0.038% of the applied dose was present in the outer layers of the *stratum corneum* (i.e. 0.026% in tape strip 1 and 0.011% in tape strip 2) with 0.012% in the remaining *stratum corneum* and only 0.088% of the applied dose present in the remaining epidermal tissue.

Table 3: Summary of cyantraniliprole distribution from the 1/20 w/w aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	0.239	0.240
Skin wash	98.7	98.9
Stratum corneum (tape strips 1-2)	0.038	0.038
Stratum corneum (tape strips 3-5)	0.012	0.012
Remaining epidermal membrane	0.088	0.088
Absorbed	0.028	0.028
Total recovered	99.0	99.2

Data excerpted from page 31 of the study report.

1/267 w/w aqueous dilution:

Mean recovery of the applied test material was 109%. Skin washing 24 hours after application removed 108% of the applied dose (Table 4). The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.697%. In terms of actual amounts this percentage equated to 0.040 µg/cm^2. The mean percentages of dose present in *stratum corneum* tape strips 1-5 were approximately 0.031%, and only 0.218% of the applied dose was present in the remaining epidermal tissue.

Table 4: Summary of cyantraniliprole distribution from the 1/267 w/w aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	<LOD	<LOD
Skin wash	6.20	108
Stratum corneum (tape strips 1-2)	0.001	0.026
Stratum corneum (tape strips 3-5)	0.0003*	0.005*
Remaining epidermal membrane	0.013	0.218
Absorbed	0.040	0.697
Total recovered	6.26	109

Values between the mean LOD and mean LOQ have been flagged with * and used as positive values
Data excerpted from page 31 of the study report.

CONCLUSION: The percentage of each dose absorbed over 24 hours was 0.003, 0.028 and 0.697% for concentrate slurry, 1/20 dilution, and 1/167 dilution, respectively. Irrespective of the applied dose, greater than 99% of the applied dose remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

A human dermal absorption factor was calculated based on the results from the in vitro rat and human skin and in vivo rat studies. The results are summarized in Table 5.

Table 5: Summary of dermal absorption of cyantraniliprole WG Formulation

Study	Sample time	Formulation concentrate **% of applied dose absorbed**	Aqueous solution (1/267 dilution) **% of applied dose absorbed**	References
In vivo--rat	24 h	0.08	1.23	MRID: 48432511
In vitro - human skin	24 h	0.003	0.679	MRID: 48432512
In vitro - rat skin	24 h	0.015	13.2	MRID: 48432513
***In vivo* Human dermal absorption factor**[a]		0.016	0.06	

[a] *In vivo* human dermal absorption factor (% absorbed) = (*in vitro* human % absorption) ×(*in vivo* rat % absorbed/ *in vitro* rat % absorption)

Revised review by US EPA (Primary Reviewer: Whang Phang, PhD).

IIIA 7.1.1 *In vitro* absorption through rat epidermis

Report: Davies D, (2011b). Cyantraniliprole/Thiamethoxam WG (A16901B) - *In Vitro* Absorption through Rat Epidermal Membranes using [^{14}C]-Cyantraniliprole. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Laboratory Report No. JV2140-REG; Syngenta File No. A16901B_10056, March 28, 2011. MRID 48432513. Unpublished.

Guidelines: OECD 428 (2004): Rat *in vitro* Dermal Absorption Study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In an *in vitro* dermal penetration study (MRID 48432513) using static glass diffusion cell system, cyantraniliprole WG formulation (A16901B: 20% cyantraniliprole and 20% thiamethoxam) penetration through the rat skin was tested. [^{14}C]-Radiolabelled cyantraniliprole doses were applied as 50% aqueous slurry of the 200 g/kg formulation concentrate (i.e. nominally 100 g cyantraniliprole/L) and as two aqueous dilutions (1/20 and 1/267 w/w) of the granular formulation concentrate. The doses were applied to epidermal membranes (derived from the back of the Wistar CrL: WI)rat) at a rate of 10 µL/cm^2 and the applications were left unoccluded for an exposure period of 24 hours. Each concentration was tested with 6 rat skin membranes. Samples of the receptor fluid (50% ethanol in water) were taken at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours during the exposure period. The distribution of cyantraniliprole within the test system and a 24 hour absorption profile were determined, using liquid scintillation counting (LSC).

The dose preparations were homogeneous both prior to and during dosing. The radiochemical purity of [^{14}C]-cyantraniliprole in the formulation concentrate slurry and the 1/20 w/w aqueous dilution, was greater than 95% both prior to and during dosing. For the 1/267 w/w aqueous dilution, the initial radiochemical purity was 92.0%, which declined slightly to 90.3% over 24 hours.

The formulation concentrate slurry: The mean recovery of the applied test material was 104%, almost all of which (104%) was recovered in the skin wash, 24 hours after application. The rate of absorption was fastest during the first hour of exposure (0.067 µg/cm^2/h), and declined to 0.003 µg/cm^2/h for the remainder of the exposure period. The overall absorption rate (0-24 hours) was 0.004 µg/cm^2/h. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.106, 0.115 and 0.126 µg/cm^2, respectively, equivalent to 0.010, 0.011 and 0.012% of the applied dose. The amount absorbed over the entire 24 hour exposure period was 0.152 µg/cm^2

equivalent to 0.015% of the applied dose. A total of 0.118% of the applied dose remained in the epidermal membrane following the 24 hour skin wash.

Aqueous dilution (1/20 w/w): The mean recovery of the applied test material was 98.0% with 95.1% of the applied dose removed by skin washing 24 hours after application. The absorption rate was fastest during the first two hours of exposure (0.425 μg/cm^2/h) after which it declined to a mean of 0.013 μg/cm^2/h for the remainder of the exposure period. The overall absorption rate (0-24 hours) was 0.028 μg/cm^2/h. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.972, 1.01 and 1.03 μg/cm^2, respectively, equivalent to 0.974, 1.01 and 1.03% of the applied dose. The amount absorbed over the entire 24 hour exposure period was 1.18 μg/cm^2 (1.18% of the applied dose). A total of 1.39% of the applied dose remained in the epidermal membrane following the 24 hour skin wash.

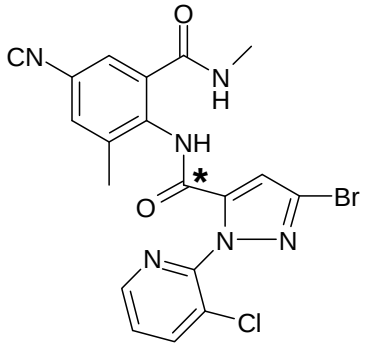
Aqueous dilution (1/267 w/w): The mean recovery of the applied test material was 107% with the majority of the applied dose (91.2%) found in the skin wash, 24 hours after application. The rate of absorption in the first three hours of exposure was 0.215 μg/cm^2/h, after which it declined to 0.013 μg/cm^2/h for the remainder of the exposure period. The mean absorption rate was 0.025 μg/cm^2/h. The amount of cyantraniliprole absorbed at 6, 8 and 10 hours was 0.721, 0.752 and 0.782 μg/cm^2, respectively equivalent to 10.1, 10.6 and 11.0% of the applied dose. A total of 2.58% of the applied dose remained in the epidermal membrane following the 24 hour skin wash. The percentage absorbed over the entire 24 hour exposure period was 13.2% of the applied dose.

The percentage of each dose absorbed over 24 hours was 0.015, 1.18 and 13.2% for concentrate slurry, 1/20 dilution, and 1/267 dilution,respectively. Irrespective of the applied dose, the majority (91.2-104%) remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Low proportions of the dose (0.118 – 2.58%) were associated with the epidermal membrane after 24 hours.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:	Cyantraniliprole
Other names:	SYN545377, DPX-HGW86-230
Description:	White powder
Lot/Batch number:	HGW86-0603-1
Purity:	93.2% a.i. w/w
Source:	Dupont Crop Protection, Stine-Haskell Research Center, P.O. Box 30, Newark, Delaware 19714-0030, USA.
Stability of test compound:	Expiry date: 24 September 2012
Unlabelled Test Material:	Thiamethoxam (CGA293343 tech.)
Description:	Light beige powder
Lot/Batch number:	SGO7K699E
Analytical reference number:	10351215
Purity:	99.0% a.i. w/w
Source:	Syngenta Crop Protection Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.
Stability of test compound:	Expiry date: End of March 2011
Radiolabelled Test Material:	[Pyrazole carbonyl-^{14}C]-Cyantraniliprole
Synonyms:	[^{14}C]-DPX-HGW86 and [^{14}C]-SYN545377
Specific activity:	50.8 µCi/mg (1.88 MBq/mg)
Radiochemical lot number:	3639107
Chemical Purity:	Not assigned
Radiochemical Purity:	Greater than 95%
Source:	Perkin Elmer Inc., 549 Albany Street, Boston, MA 02118, USA.
Stability of radiochemical:	Expiry date: Not assigned
Structure:	



* denotes the position of [^{14}C]-labelled atoms

Representative formulation tested:	Cyantraniliprole:Thiamethoxam (20%:20%) WG formulation A16901B
Blank Formulation:	A16901B Blank (milled) contained all the components of formulation A16901B, except for the active ingredients cyantraniliprole and thiamethoxam.
Reference number:	DTL reference TS00111/002/002
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.
Dose vehicle for spray dilutions:	CIPAC D water
Batch number:	CA1130R
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333, Münchwilen, Switzerland.

Relevance of Test material to Proposed Formulation: The formulation concentrate and two aqueous dilution doses (1/20 and 1/267 w/w) were prepared in accordance with instructions supplied by the Sponsor, to mimic the commercial formulation and two representative end-use dilutions.

Study Design and Methods:

Study dates: Start: August 8, 2010 End: September 28, 2010

Dose preparation: The three doses were prepared to mimic the commercial A16901B formulation and two representative aqueous end-use dilutions (1/20 and 1/267 w/w) using unlabelled cyantraniliprole, [^{14}C]-cyantraniliprole, unlabelled thiamethoxam and formulation blank. As the 200 g cyantraniliprole/kg commercial product is a dry wettable granular formulation, the formulation concentrate dose comprised a 50% aqueous slurry of the dry material, to enable homogeneous doses to be pipetted on the surface of the epidermal membranes. The doses were prepared as close to the time of application as was practicable and were analysed by HPLC to confirm their suitability for use in the study. The radioactivity content and homogeneity of each dose preparation was determined by analysing sub-samples of solvent dilutions by LSC. The mean concentrations of cyantraniliprole in the formulation concentrate slurry, 1/20 and 1/267 dilutions were 2.80 mg, 0.256 mg and 0.018 mg/25.4 µL respectively. The formulation concentrate dose was prepared on 8-9 August 2010, with analyses on 9-10 August. The 1/20 dilution dose was prepared on 16 August 2010, with analyses on 16-17 August. The 1/267 dilution dose was prepared and analysed on 23 September 2010.

Particle size assessment: Each dose was milled during its preparation to ensure that the particle size was similar to the commercial formulation. Particle size measurement was performed using a validated particle sizing procedure, based upon the measurement of particles on microscope slides fitted with a graticule, to ensure that the mean particle size of *circa* 4-6 µm had been

achieved. The formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions were particle sized on 3 August 2010, 5 August 2010 and 23 September 2010, respectively.

Diffusion cell: The type of static glass diffusion cell used in this study has an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL. Discs of approximately 3.3 cm diameter of prepared skin samples were mounted, dermal side down, in such diffusion cells held together with individually numbered clamps and placed in a water bath maintained at 32ºC ± 1ºC. Diffusion of cyantraniliprole into and across the epidermal membrane to a receptor fluid was measured during this study.

Receptor fluid: The receptor fluid (50% ethanol in water) was chosen to ensure that the cyantraniliprole would freely partition into this solvent from the skin membrane and never reach a concentration that would limit its diffusion.

Skin preparations: Epidermal membranes were prepared from clipped dorsal skin from Wistar Crl:(WI) strain male rats aged 28 days ± 2 days by chemical separation. The skins were soaked for approximately 20 hours in 1.5 M sodium bromide then rinsed in distilled water. The epidermis was carefully peeled from the dermis. Each membrane was given an identification number and stored frozen, at approximately -20ºC, on aluminium foil until required for use. Six skin membranes were employed for each test concentration.

Skin preparation integrity: Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Rat membranes with a measured resistance of <2.5 kΩ were regarded as having a lower integrity than normal and not used for exposure to the test materials, due to the possibility of compromised barrier function. Only membranes with an acceptable resistance, thereby showing that they were intact, were used in the study.

Application to the skin: Each application was represented by six intact membranes from at least two rats. Each applied dose was weighed and represented 10 µl/cm² (25.4 µl per cell) and was left unoccluded for the exposure period.

Temperature: Throughout the experiment the receptor fluid (50% ethanol in water) was stirred and the epidermal membranes were maintained at a normal skin temperature of 32 ± 1°C in a water bath.

Duration of exposure and sampling: The epidermal membrane was exposed to the test preparations for 24 hours during which time 0.5 mL samples of receptor fluid were taken at suitable intervals (pre, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours) for LSC analysis to allow adequate characterisation of the absorption profile. The receptor volume was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume, after each sample had been taken. The samples of receptor fluid were analysed by LSC.

Terminal procedures: The donor chamber was carefully removed and the underside wiped with a sponge pre-wetted with 3%Teepol L® in water which were added to the wash sponges. The donor chamber was washed with acetonitrile and the sample analysed for [^{14}C]-cyantraniliprole by LSC.

The epidermal surface of the skin was gently washed by swabbing the application site with three natural sponges pre-wetted with a solution of 3% Teepol L® in water. Following assessment of radioactivity levels on the skin surface with a Geiger counter, two sponges pre-wetted with water, were used to further swab the surface. The sponges were digested in Soluene 350® and made up to a recorded volume. A sample of the digest was taken for analysis by LSC. The epidermal membrane was carefully removed from the receptor chamber, digested in Soluene 350® and analysed by LSC. A tape stripping technique was not performed owing to the fragility of rat epidermis.

Analysis: All components of the test system (e.g. receptor fluid, skin wash, donor chamber and epidermis) were analysed by LSC and the recovery determined. Samples were analysed between the following dates. Formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions, 12-16 August 2010, 19-23 August 2010 and 24-28 September 2010, respectively.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of cyantraniliprole in the receptor solution in terms of µg/cm^2, 'percentage of dose absorbed' and rates of absorption (µg/cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg/cm^2) and 'percentage of applied dose' (see Tables below). Results presented in the report were generated using an Excel spreadsheet developed at DTL.

Definition of absorbed and unabsorbed test material: The absorbed (systemically available) dose is considered to be the cyantraniliprole detected in the receptor fluid. Material removed from the surface of the epidermis by the washing procedure is regarded as unabsorbed. Cyantraniliprole recovered from the epidermis at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study.

RESULTS AND DISCUSSION

Achieved doses: LSC analysis of the dose preparations confirmed that the achieved dose levels were 110, 10.1 and 0.712 g cyantraniliprole/L for the formulation concentrate slurry and two aqueous dilutions (1/20 w/w and 1/267 w/w), respectively. The specific activity values for [^{14}C]-cyantraniliprole in the formulation concentrate slurry, 1/20 and 1/267 w/w aqueous dilutions, expressed as dpm/µg of cyantraniliprole were 4289, 15071 and 112800 dpm/µg, respectively. The dose preparations were considered to be homogeneous and acceptable for use in this study as the percentage relative standard deviation (%RSD) for all preparations was less than 5%.

Stability of [^{14}C]-cyantraniliprole in dose preparations: Cyantraniliprole, when formulated as the formulation concentrate slurry and a 1/20 w/w aqueous dilution, was shown to be stable by HPLC for a period of time longer than that used in the study. Radiochemical purities of greater than 95% were seen in all these dose preparations both prior to application and 48 hours post dose preparation. For the 1/267 w/w aqueous dilution, the initial radiochemical purity was 92.0% which declined to 90.3% over 24 hours.

Particle size: The mean particle size range, based on the normal manufacture process for this product, was *circa* 4-6 µm. The mean particle size achieved for the formulation concentrate slurry was 5.03 µm. For the 1/20 and 1/267 w/w aqueous dilutions, the mean particle sizes achieved were 6.26 and 5.34 µm, respectively. The particle size of the formulated test materials was therefore considered to be acceptable for the purposes of this study.

Absorption from the formulation concentrate slurry:

Absorption of cyantraniliprole was fastest during the first hour of exposure. Absorption between 0-1 hour was 0.067 µg/cm^2/h after which it slowed to 0.003 µg/cm^2/h for the remainder of the exposure period (1-24 hours) (Table 1). The absorption rate between 0-24 hours was 0.004 µg/cm^2/h. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.106, 0.115 and 0.126 µg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose equated to 0.010, 0.011 and 0.012%. The amount absorbed over the entire 24 hour exposure period was 0.152 µg/cm^2 (0.015% of the applied dose).

Absorption from the 1/20 w/w aqueous dilution:

Absorption of cyantraniliprole as 1/20 w/w aqueous dilution was fastest during the first two hours of exposure (Table 1). Absorption between 0-2 hour was 0.425 µg/cm^2/h after which it slowed to 0.013 µg/cm^2/h for the remainder of the exposure period (2-24 hours). The absorption rate between 0-24 hours was 0.028 µg/cm^2/h. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.972, 1.01 and 1.03 µg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.974, 1.01 and 1.03%. The amount absorbed over the entire 24 hour exposure period was 1.18 µg/cm^2 (1.18% of the applied dose).

Table 1: Summary of cyantraniliprole absorption through rat epidermal membranes

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates		Mean Dose Absorbed		
	Time period (h)	Absorption rate ($\mu g/cm^2/h$)	Time (h)	Amount ($\mu g/cm^2$)	Percentage
Formulation concentrate slurry (n = 5) (110 g Cyantraniliprole/L)	0-1	0.067	6	0.106	0.010
	1-24	0.003	8	0.115	0.011
	0-24	0.004	10	0.126	0.012
			24	0.152	0.015
1/20 w/w Dilution (n = 6) (10.1 g Cyantraniliprole/L)	0-2	0.425	6	0.972	0.974
	2-24	0.013	8	1.01	1.01
	0-24	0.028	10	1.03	1.03
			24	1.18	1.18
1/267 w/w Dilution (n=6) (0.712 g Cyantraniliprole/L)	0-3	0.215	6	0.721	10.1
	3-24	0.013	8	0.752	10.6
	0-24	0.025	10	0.782	11.0
			24	0.938	13.2

Data excerpted from page 29 of the study report.

Absorption from the 1/267 w/w aqueous dilution:

Absorption of cyantraniliprole as 1/267 w/w aqueous dilution was fastest during the first three hours of exposure (Table 1). Absorption between 0-3 hours was 0.215 $\mu g/cm^2/h$ after which it slowed to 0.013 $\mu g/cm^2/h$ for the remainder of the exposure period (3-24 hours). The absorption rate between 0-24 hours was 0.025 $\mu g/cm^2/h$. The amounts of cyantraniliprole absorbed at 6, 8 and 10 hours were 0.721, 0.752 and 0.782 $\mu g/cm^2$, respectively. These respective amounts expressed as percentages of the applied dose were 10.1, 10.6 and 11.0% at 6, 8, and 10 hours respectively. The amount absorbed over the entire 24 hour exposure period was 0.938 $\mu g/cm^2$ (13.2% of the applied dose).

MASS BALANCE AND CYANTRANILIPROLE DISTRIBUTION
Formulation concentrate slurry:

Mean recovery of the applied test material was 104%. The vast majority of the applied dose, 104% was found in the skin wash, 24 hours after application (Table 2). The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.015%. This percentage equated to 0.152 $\mu g/cm^2$. A total of 0.118% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure.

Table 2: Summary of cyantraniliprole distribution from the formulation concentrate slurry – 24 hour exposure

Test Compartment (n=5)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	0.422	0.042
Skin wash	1052	104
Epidermal membrane	1.20	0.118
Absorbed[a]	**0.152**	**0.015**
Total recovered	1054	104

[a:] Absorbed = amount in receptor fluid. Data excerpted from page 30 of the study report

1/20 w/w aqueous dilution:

Mean recovery of the applied test material was 98.0%. Skin washing 24 hours after application removed 95.1% of the applied dose (Table 3). The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 1.18%. In terms of actual amounts this percentage equated to 1.18 µg/cm^2. A total of 1.39% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure.

Table 3: Summary of cyantraniliprole distribution from the 1/20 aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	0.318	0.318
Skin wash	94.9	95.1
Epidermal membrane	1.39	1.39
*Absorbed[a]	1.18	1.18
Total recovered	97.8	98.0

[a:] Absorbed = amount in receptor fluid. Data excerpted from page 30 of the study report.

1/267 w/w aqueous dilution:

Mean recovery of the applied test material was 107%. Skin washing 24 hours after application removed 91.2% of the applied dose (Table 4). The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 13.2%. In terms of actual amounts this percentage equated to 0.938 µg/cm^2. A total of 2.58% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure.

Table 4: Summary of cyantraniliprole distribution from the 1/267 aqueous dilution – 24 hour exposure

Test Compartment (n=6)	Mean µg Cyantraniliprole per cm²	Mean % of applied dose
Donor chamber	0.045	0.636
Skin wash	6.49	91.2
Epidermal membrane	0.183	2.58
Absorbed[a]	**0.938**	**13.2**
Total recovered	7.64	107

[a:] Absorbed = amount in receptor fluid. Data excerpted from page 30 of the study report.

CONCLUSION: The percentage of each dose absorbed over 24 hours was 0.015, 1.18 and 13.2% for concentrate slurry, 1/20 dilution, and 1/267 dilution, respectively. Irrespective of the applied dose, the majority (91.2-104%) remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Low proportions of the dose (0.118 – 2.58%) were associated with the epidermal membrane after 24 hours.

A human dermal absorption factor was calculated based on the results from the in vitro rat and human skin and in vivo rat studies. The results are summarized in Table 5.

Table 5: Summary of dermal absorption of cyantraniliprole WG Formulation

Study	Sample time	Formulation concentrate **% of applied dose absorbed**	Aqueous solution (1/267 dilution) **% of applied dose absorbed**	References
In vivo--rat	24 h	0.08	1.23	MRID: 48432511
In vitro - human skin	24 h	0.003	0.679	MRID: 48432512
In vitro - rat skin	24 h	0.015	13.2	MRID: 48432513
In vivo Human dermal absorption factor[a]		0.016	0.06	

[a] *In vivo* human dermal absorption factor (% absorbed) = (*in vitro* human % absorption) ×(*in vivo* rat % absorbed/ *in vitro* rat % absorption)